

Two Types of Functionally Distinct NAD(P)H Dehydrogenases in *Synechocystis* sp. Strain PCC6803*

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Hiroshi Ohkawa‡, Himadri B. Pakrasi§, and Teruo Ogawa‡¶

From the ‡Bioscience Center, Nagoya University, Chikusa, Nagoya 464-8601, Japan and §Department of Biology, Washington University, St. Louis, Missouri 63130-4899

The *ndhD* gene encodes a membrane protein component of NAD(P)H dehydrogenase. The genome of *Synechocystis* sp. PCC6803 contains 6 *ndhD* genes. Three mutants were constructed by disrupting highly homologous *ndhD* genes in pairs. Only the $\Delta ndhD1/\Delta ndhD2$ ($\Delta ndhD1/D2$) mutant was unable to grow under photoheterotrophic conditions and exhibited low respiration rate, although the mutant grew normally under photoautotrophic conditions in air. The $\Delta ndhD3/\Delta ndhD4$ ($\Delta ndhD3/D4$) mutant grew very slowly in air and did not take up CO₂. The results demonstrated the presence of two types of functionally distinct NAD(P)H dehydrogenases in *Synechocystis* PCC6803 cells. The $\Delta ndhD5/\Delta ndhD6$ ($\Delta ndhD5/D6$) mutant grew like the wild-type strain. Under far-red light (>710 nm), the level of P700⁺ was high in $\Delta ndhD1/D2$ and M55 (*ndhB*-less mutant) at low intensities. The capacity of Q_A (tightly bound plastoquinone) reduction by plastoquinone pool, as measured by the fluorescence increase in darkness upon addition of KCN, was much less in $\Delta ndhD1/D2$ and M55 than in $\Delta ndhD3/D4$ and $\Delta ndhD5/D6$. We conclude that electrons from NADPH are transferred to the plastoquinone pool mainly by the NdhD1-NdhD2 type of NAD(P)H dehydrogenases.

The Type I NAD(P)H dehydrogenase complex (NDH-1)¹ in cyanobacteria is involved in both the respiratory and photosynthetic electron transport chains (1). The whole genome sequence data base for *Synechocystis* sp. PCC6803 has shown the presence of genes for 12 subunits of NDH-1 with the large, hydrophobic NdhB, NdhD, and NdhF subunits being core membrane components (2). The data base also reveals that *ndhD* and *ndhF* are present as gene families with six and three members, respectively (note that NdhF4 has homology to NdhD5 and has been designated as NdhD6 (3)), although most *ndh* genes are present as single copies. This suggests that several types of NDH-1 exist in cyanobacteria, each with different NdhD and/or NdhF subunits, and with each potential complex having differing functions (4–6). In fact, of the five

ndhD-less mutants, $\Delta ndhD3$ is the only mutant that displays the phenotype of slow growth at limiting CO₂ (i.e. 50 ppm CO₂) and reduced affinity for CO₂ uptake, whereas the other *ndhD*-less mutants ($\Delta ndhD1$, $\Delta ndhD2$, $\Delta ndhD4$, and $\Delta ndhD5$) do not show such phenotype (6).

It has been demonstrated that NDH-1 is essential for inorganic carbon (CO₂ and HCO₃⁻; designated C_i) transport in cyanobacteria (3–11), and it was assumed that ATP produced by NDH-1-dependent cyclic electron flow is essential to energize the C_i transport (7, 12). However, a recent observation indicated that mutations in *ndh* genes lead to inhibition of CO₂ uptake rather than HCO₃⁻ uptake (6). This suggested that CO₂ uptake is energized differently from HCO₃⁻ uptake. The presence of an ATP-dependent HCO₃⁻ transporter in *Synechococcus* PCC7942 has been recently demonstrated (13). In an attempt to see if there are functionally distinct NDH-1 complexes, we constructed double mutants of *Synechocystis* sp. PCC6803 by disrupting highly homologous *ndhD* genes in pairs and analyzed their growth under various conditions, CO₂ uptake and redox levels of P700 and plastoquinone (PQ) pool. The results suggested the presence of two types of functionally distinct NDH-1 complexes, one essential for photoheterotrophic growth of the cells and the other essential for CO₂ uptake.

EXPERIMENTAL PROCEDURES

Growth Conditions—Wild-type and mutant cells of *Synechocystis* sp. PCC6803 were grown at 30 °C in BG11 medium (14) buffered with 20 mM Tes-KOH (pH 8.0) and bubbled with 3% CO₂ in air (v/v) or air (about 400 μl of CO₂ liter⁻¹). Solid medium was BG11-supplemented with 1.5% agar, 5 mM sodium thiosulfate, and 20 mM of the same buffer. Continuous illumination was provided by fluorescent lamps, generating photosynthetically active radiation of 60 μmol of photons m⁻² s⁻¹.

Construction of Mutants—Construction of single *ndhD* mutants, e.g. slr0331 (*ndhD1*), slr1291 (*ndhD2*), slr11733 (*ndhD3*), slr10027 (*ndhD4*), and slr2007 (*ndhD5*)-less mutants, has been described in a previous study (6). Descriptions of these mutants and the slr2009 (*ndhD6*)-less mutant have been deposited on the Web (CyanoMutants), where the drug resistance cassette used for each inactivation and the sites of insertion into the target genes are shown. The constructs used to generate the single mutants were used to transform various appropriate single mutants of *Synechocystis* sp. PCC6803 to generate the double mutants, i.e. $\Delta ndhD1/\Delta ndhD2$ ($\Delta ndhD1/D2$), $\Delta ndhD3/\Delta ndhD4$ ($\Delta ndhD3/D4$), and $\Delta ndhD5/\Delta ndhD6$ ($\Delta ndhD5/D6$). M55 is the mutant constructed by inserting a Km^r cartridge at the *Bam*HI site in *ndhB*, as described previously (7). The mutated *ndhD* genes in the transformants were segregated to homogeneity (by successive streak purification) as determined by polymerase chain reaction amplification.

Determination of Growth Characteristics—Wild-type and mutant strains grown under 3% CO₂ were collected and resuspended in fresh BG11 medium to an OD_{730 nm} of 1.0, 0.1, or 0.01. 2 μl of the cell suspensions was spotted onto BG11 agar plates buffered at various pHs in the absence or presence of 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and 5 mM glucose. The plates were incubated under 3% CO₂ in air (v/v) or air for 5 days with continuous illumination by fluorescent lamps under a photosynthetically active radiation intensity of 60 μmol of photons m⁻² s⁻¹. The OD_{730 nm} was measured using a recording spectrophotometer, model UV2200 (Shimadzu Co., Kyoto,

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¶ To whom correspondence should be addressed: Tel.: 81-52-789-5215; Fax: 81-52-789-5214; E-mail: h44975a@nucc.cc.nagoya-u.ac.jp.

¹ The abbreviations used are: NDH-1, Type I NAD(P)H dehydrogenase complex; C_i, inorganic carbon; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FR, far red; PQ, plastoquinone; SDH, succinate:quinol oxidoreductase; Tes, *N*-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; PSI, photosystem I; Q_A, tightly bound plastoquinone.

TABLE I

Amino acid sequence similarity of the NdhD subunits in *Synechocystis* sp. PCC6803

The similarity was calculated using the Clustal W-based program from DNASTar software.

	NdhD1	NdhD2	NdhD3	NdhD4	NdhD5	NdhD6
			%			
NdhD1		60.0	31.9	33.1	15.0	15.8
NdhD2			33.1	35.7	17.7	17.6
NdhD3				52.8	17.0	17.4
NdhD4					18.3	18.8
NdhD5						52.8
NdhD6						

Japan).

Assay of Respiratory Activity—Oxygen uptake was measured in the dark with a Clark type oxygen electrode (Hansatech, United Kingdom). Cells were suspended in 20 mM Tes-KOH (pH 8.0) containing 15 mM NaCl, and the activities of respiration were measured as the rates of oxygen uptake 3 min after addition of 5 mM glucose, as described by Mi *et al.* (12).

CO₂ Exchange Measurements—The wild-type and mutant cells grown under 3% CO₂ in air (v/v) were bubbled with air for 18 h in the light and then harvested. Cells were suspended in 25 ml of 20 mM Hepes-KOH buffer, pH 7.0, containing 15 mM NaCl at a chlorophyll level of 4.3 μg ml⁻¹ and placed in a reaction vessel (15, 16). CO₂ exchange of the cell suspension was measured at 30 °C using an open gas analysis system, which records the rate of CO₂ exchange as a function of time. Air containing 340 μl of CO₂ liter⁻¹ was passed into the reaction vessel at a flow rate of 1.0 liter min⁻¹, the exchanged gas was dried, and then the CO₂ concentration was analyzed using an infrared gas analyzer.

Measurements of the Redox Level of P700—Changes in levels of P700⁺ were monitored by measuring the absorbance at 820 nm on an amplitude-modulation (PAM) chlorophyll fluorometer with a dual wavelength P700 unit (ED-700DW-T), as described by Schreiber *et al.* (17). The monitoring conditions were the same as those described by Mi *et al.* (12). The oxidation of P700 was induced by illumination with far-red (FR) light (>710 nm) or a 50-ms pulse of saturating white light (1500 watts m⁻²). Wild-type and mutant cells used for P700 measurements were grown under 3% CO₂ in air (v/v) to late logarithmic phase followed by incubation in darkness for 32 h.

Chlorophyll Fluorescence Measurements—Time-based fluorescence measurements were performed on a dual modulation kinetic fluorometer (model FL-100, Photon Systems Instruments, Brno, Czech Republic) interfaced with a computer (18). The duration of the measuring flashes was 5 μs, and the measurements were performed at room temperature. Wild-type and mutant cells were harvested in the logarithmic growth phase and suspended in fresh BG11 at a chlorophyll concentration of 2 μg ml⁻¹. The fluorescence level (*F*₀) was measured for 15 s with the measuring flash of 1-s intervals, and then KCN was injected to a final concentration of 1 mM. The measuring flash was kept on at 10-s intervals for the duration of the 6-min time course. The measuring flash itself did not have a noticeable actinic effect. The fluorescence yield values were recorded as a function of incubation time with KCN.

Other Methods—Unless otherwise stated, standard techniques were used for DNA manipulation (19). Pigments in the cells were extracted in methanol, and the concentration of chlorophyll in the extract was determined (20).

RESULTS

Six *ndhD* Genes—The *Synechocystis* sp. PCC6803 genome contains six *ndhD* genes that were denoted as *ndhD1* (*slr0331*), *ndhD2* (*slr1291*), *ndhD3* (*sll1733*), *ndhD4* (*sll0027*), *ndhD5* (*slr2007*), and *ndhD6* (*slr2009*) (3). Table I summarizes the amino acid sequence similarity of the products of these genes. NdhD1, NdhD3, and NdhD5 showed the highest homology to NdhD2, NdhD4, and NdhD6, respectively. Double mutants were constructed by disrupting highly homologous *ndhD* genes in pairs, *i.e.* *ndhD1/ndhD2*, *ndhD3/ndhD4*, and *ndhD5/ndhD6*.

Growth Characteristics—To explore how the inactivation of *ndhD* genes affects the growth characteristics of the cells, growth of the mutant strains was examined under photoautotrophic conditions on solid BG11 medium buffered at various

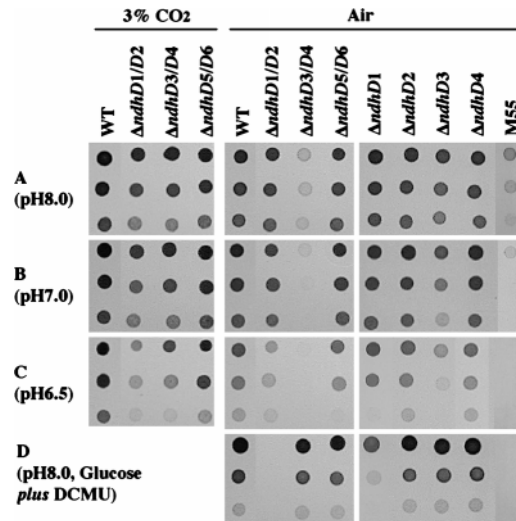


FIG. 1. Effects of pH and CO₂ concentration on the growth of wild-type and mutants on agar plates and their growth under photoheterotrophic conditions. The wild-type and mutant cells of *Synechocystis* were pelleted by centrifugation and resuspended in BG11 medium of pH 8.0, 7.0, or 6.5. Two μl of the cell suspensions, OD_{730 nm} values of 1.0 (upper rows of the panels), 0.1 (middle rows), and 0.01 (lower rows) were spotted on agar plates containing BG11 buffered at pH 8.0 (A and D), pH 7.0 (B), and pH 6.5 (C). Glucose (5 mM) and DCMU (10 μM) were added to the plates for photoheterotrophic growth (D). The plates were incubated under 3% CO₂ in air (v/v) or under air for 5 days at 60 μmol m⁻² s⁻¹ photons of photosynthetically active radiation.

pHs in the presence of 3% CO₂ or air as well as under photoheterotrophic conditions on the same medium (pH 8.0) containing 5 mM glucose and 10 μM DCMU with air. There was a striking difference between the growth characteristics of *ΔndhD1/D2* and *ΔndhD3/D4*. The *ΔndhD1/D2* mutant grew as fast as the wild-type at pH levels of 8.0 and 7.0 in air but was unable to grow under photoheterotrophic conditions (Fig. 1, middle column panels). In contrast, *ΔndhD3/D4* grew very slowly in air, although the mutant grew as fast as the wild-type under photoheterotrophic conditions. These results demonstrated that there are at least two types of functionally distinct NDH-1 complexes in *Synechocystis*. One type of NDH-1 is essential for the photoheterotrophic growth of cells, and the other type is essential for growth under low CO₂ concentrations. No significant difference was observed between the wild-type and *ΔndhD5/D6* strains in their growth characteristics under the conditions examined. Both *ΔndhD1/D2* and *ΔndhD3/D4* grew more slowly than the wild-type at pH 6.5 even under 3% CO₂ (Fig. 1, panels C). The growth of *ΔndhD5/D6* was also slower than the wild-type at pH 6.5 in air. Thus, all the *ndhD* genes appear to be needed for cells to grow under acidic conditions.

The panels on the right column in Fig. 1 show the growth of four single *ndhD*-less mutants and M55 in air under photoautotrophic and photoheterotrophic conditions. The *ΔndhD1* mutant exhibited partially the phenotype of *ΔndhD1/D2* and grew poorly under photoheterotrophic conditions. On the other hand, *ΔndhD3* exhibited slow growth in air. However, none of these single mutants showed the severe phenotypes of the double mutants *ΔndhD1/D2* and *ΔndhD3/D4*. Double mutants constructed by inactivating *ndhD* genes in different combinations did not show the phenotype of *ΔndhD1/D2* or *ΔndhD3/D4* (data not shown). As expected, M55 exhibited the phenotypes of both *ΔndhD1/D2* and *ΔndhD3/D4* and did not grow under air as well as under photoheterotrophic conditions (Fig. 1, right column panels).

Respiration—The rates of respiration of the wild-type and mutant cells in the presence of glucose are summarized in Table II. Glucose enhanced the respiration rates by 50–400%.

TABLE II

Respiration rates of wild-type and *ndh* mutants of *Synechocystis* sp. PCC6803

Cells were suspended in 20 mM Tes-KOH (pH 8.0) containing 15 mM NaCl. Oxygen consumption of the cell suspension was measured in darkness 3 min after addition of glucose (final concentration of 5 mM). Results are shown as means \pm SD ($n = 4-7$).

Mutant	Oxygen uptake
	$\mu\text{mol of O}_2 \cdot \text{mg chlorophyll}^{-1} \text{ h}^{-1}$
Wild-type	24.0 \pm 3.6
$\Delta\text{ndhD1/D2}$	6.6 \pm 2.7
$\Delta\text{ndhD3/D4}$	26.1 \pm 1.5
$\Delta\text{ndhD5/D6}$	18.9 \pm 2.9
M55	4.6 \pm 0.57

Constant rates of oxygen uptake were attained 0.5 ~ 2 min after addition of glucose and continued until oxygen in the cell suspension was exhausted. Out of the three double mutants, only $\Delta\text{ndhD1/D2}$ showed significantly reduced respiration rate, which was about one-fourth the wild-type activity but was 40% higher than that of M55. The respiration rates of $\Delta\text{ndhD3/D4}$ and $\Delta\text{ndhD5/D6}$ were not significantly different from the rate of the wild-type.

CO₂ Uptake—Fig. 2 shows changes in the rates of CO₂ exchange of the wild-type and mutant cells upon switching the light on and off, as measured using the gas analysis system (15, 16). When the suspension of the wild-type cells was illuminated, the rate of CO₂ uptake increased and reached the maximum level in 20 s. An efflux of CO₂ from the cells was observed immediately after turning the light off. Similar CO₂ exchange profiles have been observed with the wild-type cells of *Synechococcus* PCC7942 (15, 16) and *Anabaena variabilis* (21) and have been shown to reflect the CO₂ uptake in the light by a CO₂-concentrating mechanism and the release of intracellular C_i as CO₂ after darkening. The $\Delta\text{ndhD3/D4}$ mutant did not take up CO₂ at all in the light, indicating that the CO₂ uptake system is impaired in this mutant. A similar result has been observed with M55 (7) and was confirmed in this study (Fig. 2). The $\Delta\text{ndhD1/D2}$ and $\Delta\text{ndhD5/D6}$ mutants showed similar CO₂ exchange profiles to the wild-type, and their CO₂ uptake activities were not significantly different from the activity in the wild-type. The ΔndhD3 mutant showed about 30% of the CO₂ uptake activity in the wild-type under our experimental conditions (340 μl of CO₂ liter⁻¹).

Redox Level of P700 under FR Light—In cyanobacteria, NADPH produced by photosystem I (PSI) reaction donates electrons to the PQ pool via NDH-1, thus constituting NDH-1-dependent PSI cyclic electron flow (12). The donation of electrons from NADPH to the PQ pool can be observed by measuring the redox changes of P700. Fig. 3 shows the redox levels of P700 in the wild-type and mutant cells as a function of the intensity of FR light. The values in this figure are normalized based on the assumption that P700 was completely oxidized when the cell suspension was illuminated with white flash light (50- μs duration) superimposed on strong FR light (98 $\mu\text{eq m}^{-2} \text{ s}^{-1}$). When the cell suspension of M55 or $\Delta\text{ndhD1/D2}$ was illuminated with strong FR light, P700 was oxidized completely and no further oxidation of P700 was observed when the white flash light was superimposed on the FR light. In contrast, oxidation of P700 in the wild-type and other mutants was not complete with the strong FR light alone and further oxidation of P700 was observed with the flash light. P700 in M55 and $\Delta\text{ndhD1/D2}$ was highly oxidized even under weak FR light, whereas the oxidation levels of P700 in the wild-type and other mutants were low under the same conditions. At 3.3 $\mu\text{eq m}^{-2} \text{ s}^{-1}$ of FR light, the levels of P700⁺ in wild-type, $\Delta\text{ndhD1/D2}$, $\Delta\text{ndhD3/D4}$, $\Delta\text{ndhD5/D6}$, and M55 strains were 2, 56, 7, 10,

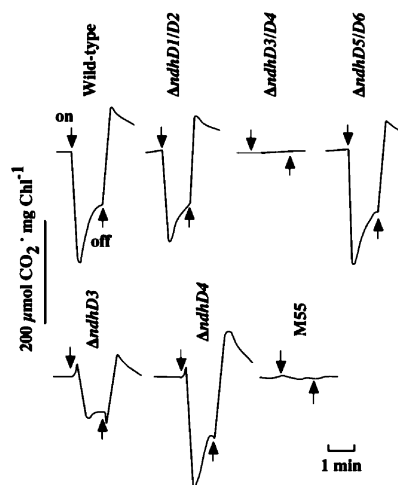


FIG. 2. CO₂ exchange of wild-type and mutant cells upon switching the light on and off, measured by an open gas analysis system. This system records the rate of CO₂ exchange as a function of time (15, 16).

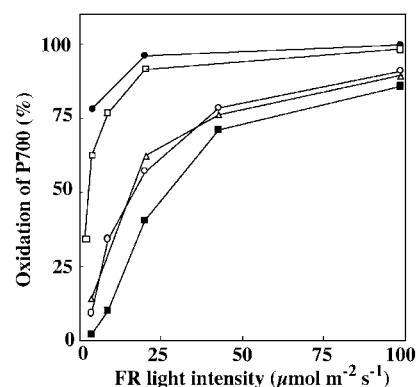


FIG. 3. Effects of the FR light intensity on the oxidation level of P700 in the wild-type and mutant cells. The P700⁺ levels were normalized assuming that P700 was completely oxidized when a 50- μs pulse of saturating white light (1500 watts m^{-2}) was given to the cell suspension superimposed to strong FR light (98 $\mu\text{eq m}^{-2} \text{ s}^{-1}$). Wild-type (■), $\Delta\text{ndhD1/D2}$ (□), $\Delta\text{ndhD3/D4}$ (○), $\Delta\text{ndhD5/D6}$ (△), M55 (●).

and 72%, respectively. Evidently, in mutants M55 and $\Delta\text{ndhD1/D2}$ the reduction of P700 is strongly inhibited and the transport of electrons from NADPH to the PQ pool, and thereby to P700, is mediated mainly by the NdhD1·NdhD2 type of NDH-1 complexes. The higher oxidation levels of P700 in $\Delta\text{ndhD3/D4}$ and $\Delta\text{ndhD5/D6}$ than in the wild-type under weak FR light indicate that the NdhD3·NdhD4 and NdhD5·NdhD6 NDH-1 complexes also have a role in mediating transfer of electrons from NADPH to the PQ pool. The capacity of electron donation from NADPH to the PQ pool by these NDH-1 complexes, however, appears to be much less than that mediated by the NdhD1·NdhD2 NDH-1 complexes.

The Redox State of the PQ Pool—The redox state of the PQ pool in thylakoids was monitored indirectly by determining the chlorophyll fluorescence yield in darkness upon addition of KCN (inhibiting oxidase activity). The fluorescence yield depends on the redox state of Q_A that is in redox equilibrium with the PQ pool. Therefore, the rate of reduction of the PQ pool can be monitored by measuring the chlorophyll fluorescence yield after oxidation of the PQ pool is blocked by KCN in the dark (22).

The wild-type cells exhibited a rapid increase in the fluorescence yield upon addition of KCN, reflecting an increase in the Q_A⁻ level due to PQ pool reduction. When the wild-type and mutant cells were illuminated in the presence of DCMU, the

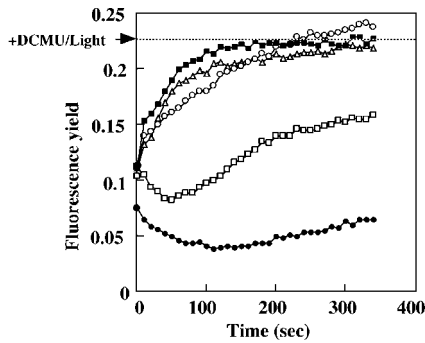


FIG. 4. Changes in chlorophyll fluorescence yield in darkness upon addition of KCN. Variable fluorescence from the wild-type, $\Delta ndhD1/D2$, $\Delta ndhD3/D4$, $\Delta ndhD5/D6$, and M55 cells was measured by very weak illumination that did not have any actinic effect. Cells were kept in darkness, and KCN (1 mM) was added at $t = 0$ s. The fluorescence yield in the presence of DCMU in the light is shown by the dotted line. Symbols for the wild-type and mutants are as in Fig. 3.

fluorescence yield increased to the level attained with the wild-type cells upon addition of KCN in the dark. The increase of the fluorescence yield was not observed with the M55 cells upon addition of KCN, indicating that NDH-1 is involved in this phenomenon. This explanation contradicts the report that the rapid increase in the fluorescence yield upon addition of KCN reflects electron donation to the PQ pool mediated by succinate:quinol oxidoreductases (SDH) (22). The *ndhD* mutants exhibited an increase in the fluorescence yield after addition of KCN, but the rate of the increase in $\Delta ndhD3/D4$ and $\Delta ndhD5/D6$ was slower than that in the wild-type strain. The rise in fluorescence yield in $\Delta ndhD1/D2$ was very slow but was much faster than in M55. Thus, the capacity of Q_A reduction by PQ pool in $\Delta ndhD1/D2$ is much less than in $\Delta ndhD3/D4$ and $\Delta ndhD5/D6$ mutants but much more than in M55. This confirms the conclusion obtained by measuring the redox level of P700 that the reduction of the PQ pool is mediated mainly by the NdhD1-NdhD2 NDH-1 complexes and that the NdhD3-NdhD4 and NdhD5-NdhD6 NDH-1 complexes also transfer electrons from NADPH to PQ pool.

DISCUSSION

The ability of cells to grow under photoheterotrophic conditions strongly depends on their respiratory activity. The $\Delta ndhD1/D2$ and M55 mutants, which exhibited low respiration rates, were unable to grow under photoheterotrophic conditions (Fig. 1 and Table II). Reduction of the PQ pool and P700 was also strongly inhibited in these mutants (Figs. 3 and 4). This indicated that the NdhD1-NdhD2 type of NDH-1 complexes mediates the transport of electrons from NADPH to the PQ pool and thereby to P700, thus constituting a PSI-dependent cyclic electron transport pathway. The NdhD3-NdhD4 and NdhD5-NdhD6 types of NDH-1 complexes also contribute to this reaction, as seen from the higher oxidation levels of P700 in $\Delta ndhD3/D4$ and $\Delta ndhD5/D6$ than in the wild-type under FR light (Fig. 3). The contribution of these complexes of NDH-1 to the PSI-dependent cyclic electron transport appears to be much smaller than that of the NdhD1-NdhD2 NDH-1 complexes (Figs. 3 and 4, Table II).

The absence of the rapid increase in the fluorescence yield in $\Delta ndhD1/D2$ and M55 upon addition of KCN suggested that this fluorescence increase reflects the electron donation to the PQ pool mediated by the NdhD1-NdhD2 NDH-1 complexes (Fig. 4). This contradicts the report by Cooley *et al.* (22) that the rapid fluorescence increase reflects the reduction of the PQ pool mediated by SDH. It appears possible that inactivation of SDH led to decreased formation of NADPH, thereby decreasing the NDH-1-dependent reduction of the PQ pool. Adversely, inacti-

vation of NDH-1 might lead to the decrease of succinate formation. Inactivation of *ndhB* or *ndhD1* plus *ndhD2* had much stronger effect on the fluorescence increase than the inactivation of *sll1625* plus *sll0823* (Fig. 4; also see Fig. 6 in Ref. 22), and hence, we prefer the former possibility.

Among the single and double *ndhD* mutants constructed in this and previous studies (5, 6), only $\Delta ndhD3/D4$ showed the accentuated phenotype of M55 (*i.e.* complete loss of CO_2 uptake activity). It is evident that inability of these mutants to grow under low CO_2 conditions is a result of inactivation of their CO_2 uptake activity (Figs. 1 and 2) and that only the NdhD3-NdhD4 NDH-1 complexes are essential for CO_2 uptake. We have previously proposed a model in which ATP produced by NDH-1-dependent PSI cyclic electron transport drives the "transport" of CO_2 (23). However, inactivation of *ndhD1* and *ndhD2*, which are essential to the PSI cyclic electron transport, did not have any significant effect on CO_2 uptake (Fig. 2). Thus, this previous model appears to be unlikely.

The mechanism of CO_2 uptake and the role of NdhD3-NdhD4 NDH-1 complex types in CO_2 uptake are not known. The CO_2 uptake reaction is postulated to be an energy-dependent unidirectional conversion of CO_2 to HCO_3^- (11, 24). Kaplan and Reinhold proposed a model in which an "alkaline pocket" produced in the light functions as a converter of CO_2 to HCO_3^- (11). The information presented in this paper does allow for limited speculation on how a particular type of NDH-1 complex might be specifically involved in CO_2 uptake. A vectorial carbonic anhydrase-like reaction could be closely associated with the NdhD3-NdhD4 NDH-1 complex type such that OH^- ions might be produced in a "localized pocket" and used to drive the conversion of CO_2 to HCO_3^- . Such a pocket could be produced at the site(s) of protonation of NADP and/or PQ, and a relatively high electron transfer rate would be required to account for the high CO_2 uptake rate. The low rate of electron transfer from NADPH to PQ pool in the $\Delta ndhD3/D4$ mutant would suggest that the NdhD3-NdhD4 NDH-1 complexes donate electrons mainly to an alternate electron acceptor. Further studies are in progress to identify the electron acceptor.

In a previous paper, the following phenotypes were reported to characterize the *ndhB*-less mutant (M55) of *Synechocystis* sp. strain PCC6803 (7). 1) It grows very slowly under air and is unable to take up CO_2 , 2) it is unable to grow under photoheterotrophic conditions, and 3) it has a low respiration rate. The first phenotype was observed in the $\Delta ndhD3/D4$ mutant described in this study and the second and third phenotypes were found for the $\Delta ndhD1/D2$ mutant (Figs. 1 and 2, Table II). Thus, it appears evident that there are two types of functionally distinct NDH-1 complexes in *Synechocystis* sp. PCC6803, and M55 exhibits the phenotype of both types of the mutants. The presence of the homologues of the six *ndhD* genes in the genome of *Anabaena* sp. PCC7120 (the Web, CyanoBase) and *Synechococcus* sp. PCC7002 (4) strongly suggests that the presence of the two types of functionally distinct NDH-1 complexes is common among various strains of cyanobacteria.

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