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NADH AND NADPH AS ELECTRON DONORS TO RESPIRATORY AND PHOTOSYNTHETHIC ELECTRON TRANSPORT IN THE BLUE-GREEN ALGA, *APHANOCAPSA*

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In the blue-green alga, *Aphanocapsa*, light inhibits respiration. This can be observed with spheroplasts when O_2 uptake is measured with NADH or NADPH as electron donor. However, NAD(P)H oxidation is unaffected by illumination. Furthermore, it was possible to demonstrate electron transfer from NAD(P)H to Photosystem I. Thus, the inhibition of respiratory oxygen uptake by light is explained by a competition of cytochrome oxidase and Photosystem I for reduction equivalents. Based on studies with inhibitors, electron transfer from NAD(P)H to Photosystem I involves the chloroplast cytochrome b_6-f complex.

In contrast to the respiratory electron-transport chain in blue-green algae, the photosynthetic electron transport processes are well understood and have been shown to be very similar to those in eukaryotic algae and higher plants [1]. Nevertheless, blue-green algae lack specific organelles, such as chloroplasts and mitochondria, in which the electron-transport reactions are normally localized. Since the early observation of light inhibition of respiration [2,3], it has been proposed that photosynthetic and respiratory electron transport might share common components [4]. Models to explain this interaction have ranged from one in which competition for ADP as a substrate of phosphorylation by both energy-conserving processes might occur [5] to one in which the two electron-transport processes are linked via common redox carriers [6].

In this work, studies of electron donation by reduced pyridine nucleotides to the respiratory and photosynthetic electron-transport chain of a blue-green alga are described. NADH and NADPH can serve as electron donors for respiratory oxygen reduction as well as for photosynthetic electron transport through Photosystem I (PS I). Inhibitor studies indicate that NAD(P)H oxidoreductase mediates electron transfer into the photosynthetic electron-transport chain at the level of plastiquinone. A light-inhibition of respiration could be demonstrated, and the mechanism of this reaction is considered in relation to a common group of carriers for respiration and photosynthesis and an electron drain through PS I in the light.

Cultivation of *Aphanocapsa* strain 6714 was carried out as previously described for other blue-green algae [7]. Growth temperature was increased to 30°C and light intensity was decreased to 25 W/m^2.

Spheroplasts were prepared using the modified method of Spiller [8]. Cells from 500 ml of suspension were collected in the late log phase by centrifugation (8000 × *g*, 30 min). The pellet was washed with 0.5 M sucrose, 10 mM $MgCl_2$ and 0.2%

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Abbreviations: PS I, photosystem I; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethyl urea; CCCP, carbonyl cyanide *m*-chlorophenyl-hydrazone; DNP-INT, 2-iodo-6-isopropyl-3-methyl-2′,4,4′-trinitro diphenyl ether.

bovine serum albumin in 20 mM potassium phosphate buffer (pH 6.5). After resuspending in the same medium, lysozyme (5 mg/ml) was added and the suspension incubated at 37°C for 90 min. The resulting spheroplasts were collected by centrifugation at 700 × *g* for 5 min. The pellet was resuspended in the above medium, from which the serum albumin was omitted. The supernatant of the last centrifugation step should be light green to light blue in color, and the percentage of spheroplasts to intact cells should be greater than 90%, as measured by acetone versus hot methanol-extracted chlorophyll [9].

Oxygen uptake by spheroplasts shocked in the reaction medium that contained 10 mM potassium phosphate, 3 mM NaCl, 10 mM $MgCl_2$, 10 mM sucrose and 20 mM Tricine-KOH buffer (pH 7.8) was measured in a Rank electrode at 20°C. The samples were illuminated with light passing through a 530 nm cut-off filter (Corning CS 3-68) at an intensity of 170 W/cm^2. Oxidation of NADH or NADPH at 25°C was monitored spectrophotometrically at 430 nm in a Gilford single beam spectrophotometer.

Aphanocapsa 6714 was chosen for the present studies because of its high rate of respiration, which exceeds the values obtained with cell-free preparations [10,11] or intact cells [12–14] of most other blue-green algae. *Aphanocapsa* also shows pronounced light inhibition of respiration which can conveniently be measured in cells in the presence of DCMU (data not shown) or with shocked spheroplasts using either NADH or NADPH as substrate (Table I).

NADH oxidation with oxygen as electron acceptor is dependent on ADP and can be increased by the uncoupler, carbonyl cyanide *m*-chlorophenyl-hydrazone (CCCP). This suggests that the respiratory reactions are coupled to ATP formation in our spheroplast preparations (see also Ref. 10). Respiration in *Aphanocapsa* spheroplasts is partially sensitive to KCN, while the presence of superoxide dismutase and catalase did not affect oxygen uptake.

Comparison of the rates of respiration with NADH and NADPH as electron donors indicate that the latter is a better substrate (Table II). However, no effect of light on reduced pyridine nucleotide oxidation was observed. Without added pyridine nucleotides, a substantial basal rate of NAD(P)H oxidation and oxygen uptake could be observed, due to endogenous NAD(P)H levels [15].

Concurrent measurement of NADH and NADPH consumption in addition to oxygen uptake showed that light did not affect the rates of pyridine nucleotide oxidation to the same extent as did oxygen uptake (Tables I and II). From this observation it can be concluded that electrons originating from the oxidation of NAD(P)H are being removed from the respiratory chain in the light somewhere between the NAD(P)H oxidoreductase system and cytochrome oxidase. This view is further substantiated when the stoichiometries of NAD(P)H oxidation and O_2 reduction are compared. As 2 mol of pyridine nucleotide are necessary for the reduction of O_2 to $2H_2O$, NADH and NADPH oxidation should be twice as fast as O_2

TABLE I

NAD(P)H-DEPENDENT OXYGEN UPTAKE OF SHOCKED *APHANOCAPSA* SPHEROPLASTS

The reaction medium (methods section) contained 0.3 mM NADH and 1 mM ADP. Values are μmol O_2/mg chlorophyll per h.

Assay conditions	Light	Dark
Complete	4.9	20.1
−ADP	0	8.7
−ADP, +CCCP (10 μM)	7.2	22.9
+DCMU (5 μM)	12.6	22.5
+Superoxide dismutase (300 units/ml) and catalase (100 μg/ml)	6.6	21.2
+KCN (1 mM)	0.8	10.9
−NADH, +NADPH (0.3 mM)	15.2	27.1

TABLE II

OXIDATION OF NADH AND NADPH BY SHOCKED SPHEROBLASTS FROM *Aphanocapsa*

The reaction medium (see methods section) contained 0.3 mM NADH and 1 mM ADP. Values are μmol oxidised/mg chlorophyll per h.

Assay conditions	−DCMU		+DCMU	
	Light	Dark	Light	Dark
−NADH, −NADPH	11.6	14.3	9.7	9.7
+NADH	36.5	38.6	30.5	34.2
+NADPH	51.9	62.1	46.4	45.8

reduction. Indeed, this is the case for respiration in darkness (compare Tables I and II). In light, however, the ratio of NAD(P)H oxidized versus O_2 reduced is much higher than 2, indicating that some of the electrons from NAD(P)H oxidation are channeled away from respiration into another pathway.

In addition to transferring electrons to O_2 in the dark, NADH and NADPH can serve as electron donors for methyl viologen reduction in the light (measured as O_2 uptake) (Table III). A certain rate of oxygen consumption occurs in the dark, contributed to by terminal respiration. In the case of NADPH as substrate, there is an additional portion of dark reduction of methyl viologen from NADPH mediated by the back reaction of ferredoxin-$NADP^+$ reductase (data not shown). The light-dependent oxygen-uptake reaction is inhibited by 2-iodo-6-iso-propyl-3-methyl-2',4,4'-trinitrodiphenylether (DNP-INT), a known inhibitor of electron transfer through the photosynthetic cytochrome *b–f* complex in algae [16], and the uncoupler CCCP stimulates the reaction. Antimycin A, an inhibitor of the mitochrondrial cytochrome *b–c* complex [17], at concentrations up to 20 μM, had no effect on light-dependent O_2 uptake. As shown in Fig. 1, electron transfer to methylviologen from NADH saturates at an NADH concentration of 0.5 mM and a K_m value of 0.2 mM was calculated.

A similar electron transport system in which PS

TABLE III

METHYL VIOLOGEN-MEDIATED OXYGEN UPTAKE WITH NADH AND NADPH AS SUBSTRATES BY SHOCKED SPHEROBLASTS FROM *APHANOCAPSA*

The reaction mixture contained NADH or NADPH (0.25 mM), methyl viologen (0.25 mM), sodium azide (0.25 mM), CCCP (10 μM) and DCMU (10 μM) in the medium described in the methods section. The rate without NAD(P)H was 7.4. Values are μmol O_2/mg chlorophyll per h.

	Substrates	
	NADH	NADPH
Complete (illuminated)	23.6	34.7
+ DNP-INT (2 μM)	15.1	21.0
− CCCP	5.9	28.5
+ Antimycin A (20 μM)	22.4	34.2
Dark	8.5	25.7

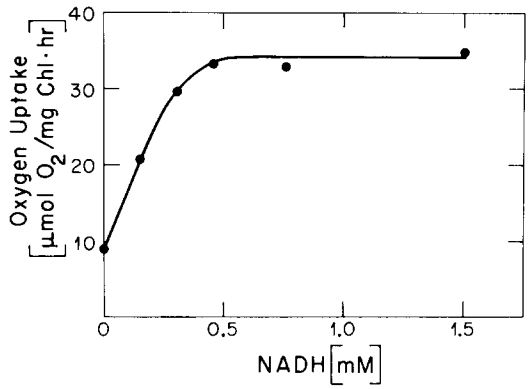

Fig. 1. Methyl viologen-mediated oxygen uptake in the light as a function of NADH concentration. The assay mixture consisted of 0.25 mM methyl viologen, 0.25 mM sodium azide, 20 μM DCMU, 10 μM CCCP, 10 mM potassium phosphate, 3 mM NaCl, 10 mM $MgCl_2$, 10 mM sucrose, 20 mM Tricine-KOH buffer (pH 7.8), and *Aphanocapsa* spheroplasts equivalent to 15 μg chlorophyll (Chl) per ml. NADH was added at the indicated concentration.

I was reduced by NADH was reported recently for the green alga *Chlamydomonas* [18,19]. Furthermore, chlorophyll fluorescence kinetic measurements with a PS I-free *Chlamydomonas* mutant suggested a coupling of NADH and NADPH oxidation to plastoquinone [20]. Recently, a competition between reducing equivalents originated from respiration and electrons from PS II for the same site could be demonstrated for *Anabaena* [21].

The data presented here demonstrate a link between respiratory and photosynthetic electron transport. Obviously, NADH and NADPH are oxidized involving the cytochrome b_6-*f* complex and serve then as an electron donor for PS I in illuminated *Aphanocapsa*. These reactions might have their physiological significance in providing an adequate electron supply for PS I in the light. Such a situation could exist if the ratio of PS I to PS II is greater than unity, as has recently been determined in *Synechococcus* [22]. Whether this situation exists in other blue-green algae remains to be determined.

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