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RESPIRATION IN ENERGY-TRANSDUCING MEMBRANES OF THE THERMOPHILIC CYANOBACTERIUM *MASTIGOCLADUS LAMINOSUS*

I. RELATION OF THE RESPIRATORY AND PHOTOSYNTHETIC ELECTRON TRANSPORT

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The thermophilic cyanobacterium *Mastigocladus laminosus* was grown at different CO_2 concentrations and temperatures. Respiratory and photosynthetic electron transport in isolated membranes were measured and their activities were compared. Cells grown at low CO_2 concentration showed respiratory electron transport, whereas Photosystem-II-dependent transport was optimal in cells grown at high CO_2 concentrations. The respiratory electron transport from NADH and succinate were KCN-sensitive, whereas NADPH-dependent O_2 uptake was not. It could be shown that NADH and succinate donate electrons in the photosynthetic electron pathway via Photosystem I. In cytochrome-*c*-553-depleted membranes added cytochrome *c*-553 could stimulate photosynthetic and respiratory electron transport. A common electron transport pathway between the quinone and cytochrome *c* is postulated.

Introduction

Mastigocladus laminosus is a thermophilic cyanobacterium with growth temperatures up to 65°C. This alga is ubiquitous and adapted to a broad spectrum of environmental conditions [1,2]. The prokaryotic cells show oxygenic photosynthesis with a mechanism which is very similar to that found in chloroplast thylakoids of eukaryotic cells and, in the dark, they exhibit respiration [3].

The respiratory electron transport chain from dehydrogenase to cytochrome *c* to cytochrome oxidase and the photosynthetic electron transport chain serving the two photosystems have basically the same components with similar structures: a quinone pool, a cytochrome *b/c* or a cytochrome *b/f* complex, and a soluble cytochrome *c*. In cyanobacteria, the thylakoids are known to be the site of the photosynthetic electron transport, whereas the localization of the respiratory chain is still controversial. The latter may be located on the plasmalemma as in other bacteria or on the thylakoid membranes sharing a common electron transport chain with the photosynthetic system. An extensive review of this topic has been published recently [6].

It was found that temperature and pH [3], as well as the supply of CO_2 during growth [4,5] can have marked effects on the physiology of cyanobacterial cells. In the present work the relation of the respiratory and photosynthetic electron transport in membranes from *M. laminosus* cells grown at different CO_2 concentrations and different tem-

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Abbreviations: Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)-ethyl]glycine; DCMU, 3-(3′,4′-dichlorphenyl)-1,1-dimethylurea; TMPD, *N,N,N′,N′*-tetramethylphenyldiamine.

peratures are investigated. Possible common electron-transport pathways are discussed.

Materials and Methods

Culture. M. laminosus strain Rr has been isolated from a hot spring in Sudur Reykir, Iceland [1]. The cells were cultivated in 10 l flasks in medium D of Castenholz [7], pH 8.2 at 40 or 50°C. Cell growth caused an increase of the pH to 8.5–8.7. The cultures were illuminated with 16 fluorescence lamps (Sylvania warm white, 25 W) with an intensity of 15 W/m^2 at the surface of the bottle. Aeration and turbulence were produced with compressed air (4 l/min) enriched with 0–5‰ CO_2 (CO_2 concentration in the air, 0.4‰).

The cells were grown for 24–48 h (early logarithmic phase), harvested in a Westfalia separator, washed twice in distilled water and stored at −20°C before use. Depending on the CO_2 concentration in the compressed air, the doubling time was 15–25 h at 40° and 8–14 h at 50° with yields of 0.3–0.5 g wet wt. per l. at 40°C and 0.5–1 g at 50°C.

The protein content of the cells was constant at all CO_2 concentrations (29–30 mg protein per g cell wet wt), whereas the chlorophyll content varied from 1.5 mg chlorophyll per g cell wet wt. at 0.4‰ CO_2 up to 2.6 mg chlorophyll per g cell wet wt. at 3‰ CO_2. Therefore, all specific activities reported here have been calculated on a protein basis.

Membrane preparation. For the preparation of spheroplasts, cells grown at 40°C with 0.4–1‰ CO_2 were used, unless stated otherwise. The cells were thawed, washed in distilled water and resuspended in a buffer comprising 40 mM Tricine-NaOH (pH 7.6)/5 mM $MgCl_2$/10 mM KCl (Tricine-$MgCl_2$-KCl buffer)/0.3 M sucrose, giving a concentration of 0.3 mg chlorophyll per ml. After the addition of lysozyme (5 mg per ml), the suspension was incubated for 90 min at 45°C and then centrifuged for 5 min at $1000 \times g$. The spheroplasts were shocked osmotically for 15 min at 4°C by resuspending in Tricine-$MgCl_2$-KCl buffer without sucrose (0.1 mg chlorophyll per ml). The membranes were then washed twice in the same buffer (centrifugation, 3 min at $1000 \times g$) taken up in Tricine-$MgCl_2$-KCl buffer with sucrose (1 mg chlorophyll per ml) and stored in ice. Where indicated, these membranes were depleted of cytochrome *c*-553 by sonication for 2 min in a Braun sonifier at maximal power.

Assay. Oxygen evolution and uptake were measured with a Clark type oxygen electrode (Rank Brothers). The reaction vessel was temperature-controlled (45°C, if not stated otherwise) and illuminated with two 150 W tungsten lamps through 10 cm of a 5% $CuSO_4$ solution. The light intensity in the cuvette was 75 W/m^2. The reaction mixture contained 2 ml Tricine-$MgCl_2$-KCl buffer with sucrose, membranes (50–100 μg chlorophyll) and the indicated additions in the following concentrations: 4 mM NADH/1 mM ferricyanide/1 mM KCN/1 μM DCMU.

Activities. In order to compare the specific activities of the different electron transport pathways in the dark and in the light, all activities are expressed in nequiv. or μequiv. electrons (e^-) per mg protein per min, taking the following electron to O_2 stoichiometries: (i) Four electrons per O_2 taken up by the respiratory system and in the

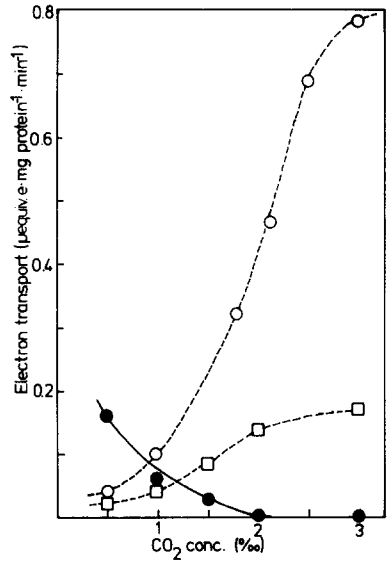

Fig. 1 Influence of CO_2 concentration during cell growth on the oxidative and light-driven electron transport in isolated membranes. Cells were grown as described in Materials and Methods with the CO_2 concentrations indicated. Membrane preparation and electron transport measurements were done as described in Materials and Methods. Dark reaction (KCN sensitive): NADH to O_2 (O_2 uptake, ●———●). Light reactions (DCMU sensitive): H_2O to O_2 (O_2 uptake, □-----□) and H_2O to ferricyanide (O_2 evolution, ○------○).

endogenous light-driven electron transport. The latter activity evolves one O_2 per four electrons in Photosystem II but takes up two O_2 per four electrons in Photosystem I, giving a net O_2 uptake of one O_2 per four electrons. Catalase activity was checked with the addition of 100 μM Na-azide and was found to be neglectable. (ii) Four electrons per O_2 evolved by the Hill reaction with ferricyanide as electron acceptor. (iii) Two electrons per O_2 taken up by the light-driven electron transport with exogenous electron donors and DCMU blocked Photosystem II.

All activities given in this work represent an average of at least three experiments.

Chlorophyll-*a* concentration was determined after extraction with 80% acetone using an extinction coefficient of 80.04 $mM^{-1} \cdot cm^{-1}$ at 663 nm. Protein determination was done according to Peterson [8]. Cytochrome *c*-553 was isolated from *Aphanizomenon flos aquae* [9].

All reagents were of the best grade available. 2-iodo-6-isopropyl-3-methyl-2′,2,4′-trinitrophenyl-ether was a gift of Dr. Oettmeier, Bochum.

Results

Influence of the growth conditions

Early observations have indicated that the availability of CO_2 to the cells – dependent on pH, CO_2 concentration, aeration rate and turbulence – greatly influences the color of cyanobacterial cells (change of the relative contents of chlorophyll, carotenoids and phycobiliproteins) as well as photosynthetic and respiratory activities [3]. In order to obtain more information on these interrelations, cells were cultivated at low (0.4‰) and high (3‰) CO_2 concentration, respectively.

The absorption spectra of whole cells show that the phycobilin-to-chlorophyll ratio is about 10-times higher in high CO_2 grown cells then in low CO_2 grown cells. In contrast to that, the carotenoid-to-chlorophyll ratio increases with decreasing CO_2 concentrations. Thus, at high CO_2 concentrations the cells appear blue-green whereas at low concentrations they are brown.

The CO_2 concentration during growth of the cells also has a marked influence on the electron transport in isolated membranes. Fig. 1 shows that the dark respiratory electron pathway with NADH as electron donor (KCN sensitive) is only active in membranes prepared from low CO_2 grown cells. On the other hand, the Photosystem II involving, light-driven electron transport activities (DCMU sensitive) are almost nil in membranes of low CO_2 grown cells and increase strongly with increasing CO_2 concentrations. The light-driven electron transport in membranes involving Photosystem I only (DCMU insensitive) is not dependent on the CO_2 concentration during growth (data not shown). Thus, in experiments where light and dark activities were studied in the same membranes, cells grown at 1–1.5‰ CO_2 were used.

The respiratory and photosynthetic electron transport systems are not the only activities exhibiting oxygen uptake, since any autooxidations of some unknown compounds may lead to oxygen consumption. Therefore, only activities which are inhibited with known system-specific inhibitors, i.e., KCN for respiration and DCMU for the Photosystem-II-dependent activities, can definitely be designated as respiratory and photosynthetic electron transport activities. Non-specific activities have been subtracted for all the data given in this paper.

The growth temperature of the cells influences the electron transport activity in isolated membranes. The dark respiratory activity is about 20% higher in cells grown at 40°C than at 50°C, whereas the light-driven photosynthetic activity is 50% higher in cells grown at the higher temperature,

Characterization of the electron-transport activities

Earlier work [2] showed that the temperature optimum of the light-driven electron transport in isolated membranes is about 40–45°C, and this was later confirmed for the dark KCN-sensitive electron transport from NADH to O_2. In contrast to this, the KCN-insensitive activity has an optimum of about 70°C. This is another indication that the KCN-insensitive O_2 uptake is not a respiratory activity.

If the respiratory and photosynthetic electron transports are indeed localized in the same membranes, a common pathway for both systems could be postulated. A typical experiment to probe this relation is shown in Fig. 2. The active endogenous oxygen uptake with H_2O as electron donor shows that the Photosystem I can efficiently donate elec-

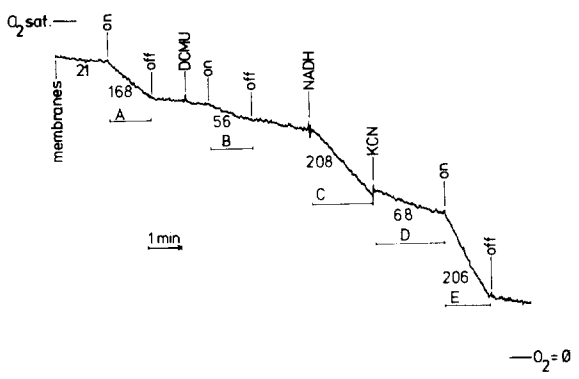

Fig. 2 Oxygen uptake in the dark and in the light. Direct recording of the oxygen electrode showing the effect of the addition of the electron donor NADH and the inhibitors KCN and DCMU to membranes of cells, grown at 1‰ CO_2. Details are given in Materials and Methods. Activities are expressed as nequiv. e^- per mg protein and min.

trons directly to O_2 (A) (Mehler reaction). This Photosystem-II-dependent reaction is inhibited to 80% with DCMU (B). The addition of NADH in the dark reveals a net respiratory electron transport of 187 nequiv. e^- per mg protein per min (C), which is inhibited to 78% by KCN (D). When the light is turned on, again a net electron transport of 185 nequiv. e^- per mg protein per min can be measured (E). This demonstrates that there is an inhibitor-insensitive movement of electrons from NADH to O_2 via Photosystem I.

TABLE I

EFFECT OF ELECTRON TRANSPORT INHIBITORS IN THE DARK AND IN THE LIGHT

Details are given in Material and Methods. All the experiments were done in the presence of 1 μM DCMU. (100% = 108 nequiv. e^- per mg protein per min in the dark and 140 nequiv. e^- per mg protein per min in the light). DNP-INT, 2-iodo-6-isopropyl-3-methyl-2′,2,4′-trinitrophenylether.

Conditions	Electron transport NADH to O_2 (%)	
	Dark	Light
Control	100	100
Inhibitors added		
1 mM KCN	26	96
1 mM sodium azide	60	75
30 μM antimycin A	68	59
100 μM rotenone	56	77
10 μM DNP-INT	75	86

Table I indicates that KCN, as a specific respiratory inhibitor, does not influence the light reaction, which confirms the experiment described in Fig. 2. Thus, the terminal oxidase of respiration is not active in the light and electrons are preferentially transferred to Photosystem I. Azide does not act as specifically as KCN, i.e., respiration is inhibited to only 40%, and electron transport in the light is also influenced in an unknown way. Antimycin A, rotenone and 2-iodo-6-isopropyl-3-methyl-2′,2,4′-trinitrophenylether are not very efficient inhibitors, but they all inhibit the electron transport to a similar extent either in the dark or in the light (Table I).

NADH is not the only electron donor to donate electrons in both the dark and in the light. Table II summerizes the activities of three other electron donors: NADPH, succinate and TMPD-ascorbate. These results show that the reaction with succinate is about half as active as that with NADH. Both activities are inhibited by KCN. On the other hand, the activity with NADPH is low and is almost insensitive to KCN. This suggests that NADPH cannot function as an electron donor to the respiratory or photosynthetic electron transport. TMPD-ascorbate is able to support electron transport in the dark and in the light.

Photosynthetic membranes of cyanobacteria can be depleted of soluble cytochrome *c*-553 by dilution and sonication. Electron-transport activities can be reconstituted in the light and in the dark by

TABLE II

ELECTRON DONORS FOR THE OXIDATIVE AND LIGHT-DRIVEN ELECTRON TRANSPORT

Details are given in Materials and Methods. All experiments were done in the presence of 1 μM DCMU. Numbers in parenthesis are activities in the presence of 1 mM KCN, given in percentage of the noninhibited reaction.

Electron donors	Activity (nequiv. $e^- \cdot mg\ protein^{-1} \cdot min^{-1}$)		
	Dark		Light
	− KCN	+ KCN	
NADH	172	52 (30%)	188
Succinate	80	20 (25%)	84
NADPH	64	48 (79%)	80
TMPD-ascorbate	164	57 (35%)	194

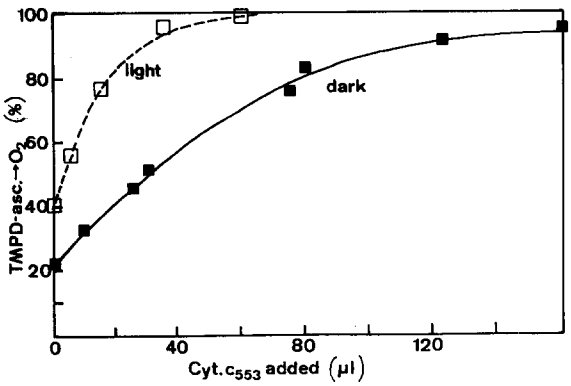

Fig. 3 Reconstitution of depleted membranes with cytochrome *c*-553 in the dark and in the light. Sonicated membranes were depleted by dilution (1 : 10) and 3 min sonication. Reconstitution was done with cytochrome *c*-553 isolated from *A. flos-aquae*. The activities were measured in the presence of 1 mM TMPD and 1 mM ascorbate. Dark reaction (■———■), 100% = 180 nequiv. e^- per mg protein per min; light reaction (□----- —□), 100% = 330 nequiv. e^- per mg protein per min.

adding again cytochrome *c* as shown in Fig. 3. The rest activities in depleted membranes (20% in the dark and 40% in the light) might be due to remaining cytochrome *c* or to TMPD-ascorbate driven, yet cytochrome-independent electron flow. The titration of these cytochrome-*c*-depleted membranes with added cytochrome *c*-553. of *A. flos-aquae* gives almost 100% reconstitution in both systems. The dark reaction is to 70% KCN sensitive and needs about 4-times more cytochrome than the light reaction. These experiments demonstrate that cytochrome *c*-553 is both an electron donor to the cytochrome oxidase in the dark and to the reaction center of Photosystem I in the light. Similar results, although with lower activities, were obtained with NADH as electron donor.

Discussion

It is known that at low CO_2 concentrations cyanobacteria can degrade their phycobiliproteins and use them as carbon source [4]. At the same time, the chlorophyll content decreases and the carotenoid content increases. Under these conditions, where not enough CO_2 is present to support growth, not all the light energy is used for photosynthesis. Thus, most of the phycobiliproteins are not necessary as antenna pigments, and so they function as carbon or nitrogen reserves. The increased amount of carotenoids probably preserves the cells from photobleaching [10].

Besides these pigment changes, other reactions are also regulated by the CO_2 concentrations. According to the results presented in this paper, CO_2 concentration regulates the electron transport in the light as well as in the dark. It is feasible that under low CO_2 conditions, where the formation of redox equivalents for CO_2 assimilation is saturated, light is solely used to drive phosphorylation in a bacterial-like cyclic electron transport with Photosystem I, while Photosystem II is shut off or degraded [11]. The inactivation of Photosystem II at low CO_2 concentrations has been reported from chloroplasts of higher plants [12]. Under these low CO_2 conditions, respiration is high, whereas at high CO_2 concentrations, respiration is much reduced, suggesting alternative dark-energy metabolism such as fermentation [13]. Still, one must remember that all these activities described here are measured in isolated membranes, so it cannot be excluded that artefacts are created during the membrane preparation.

Several data presented here suggest common components for the respiratory and photosynthetic electron transport on the thylakoid membranes: (i) NADH and succinate serve as electron donors with similar activities in the dark and in the light. This suggests that the same dehydrogenases are active in both systems and can feed electrons to the common pathway. (ii) Electron transport inhibitors (Antimycin A and 2-iodo-6-isopropyl-3-methyl-2′,2,4′-trinitrophenylether) which react with complexes of the common pathway have a similar effect in the dark and in the light. (iii) The depletion and reconstitution of cytochrome *c*-553 in dark respiration and in light-dependent photosynthetic electron transport demonstrates that cytochrome *c*-553 can donate electrons either to the oxidase or to the reaction center of Photosystem I. This is in agreement with results which were reported earlier [14,25]. A possible arrangement of the reactions in the cyanobacterial membrane is illustrated in the review published earlier [6].

Based on various experiments with quinones and cytochromes, similar common pathways have been suggested in other cyanobacteria as is sum-

merized in a recent review [6]. Evidence for a respiratory chain has even been described in chloroplasts of eucaryotic cells [15,16]. This could be another indication that chloroplasts are derived from endosymbiotic cyanobacteria which originally contained a respiratory system. As reported elsewhere [17] it is possible that respiration is located not only on the thylakoid membranes but also on the plasmalemma as in other respiring bacteria. The differentiation of plasmalemma activities versus thylakoid activities would require the separation of the two membrane types. Although there is a report of such a preparation , no activity measurement were done [18].

The KCN sensitivity of respiration suggests the existence of a mitochondrial type cytochrome oxidase [19]. KCN insensitive oxygen uptake in the dark might originate from alternative oxidases [20] or from autooxidation reactions, e.g., the oxidation of NADPH by the ferredoxin-NADPH oxidoreductase (diaphorase) [21]. DCMU insensitive endogenous photooxidations are also considered here as non-specific reactions. One cannot exclude the possibility, that part of the light-driven NADH oxidation might be an oxidation which is not related to the photosynthetic electron transport chain, since no inhibitor is able to block this activity more than 50%.

In the membranes described here, NADH is a more effective electron donor than NADPH for the electron transport in the dark as well as in the light. This is in agreement with results from other cyanobacteria [21,22]. On the other hand, there are organisms where NADPH seems to be more effective than NADH [20,23]. This discrepancy may be due to different activities of the transhydrogenase or the specificity of the dehydrogenase(s).

A proof for true respiratory activities in these cell-free membranes is the fact that oxidative phosphorylation can be measured parallel to the NADH-dependent electron transport. These results are presented and discussed in the subsequent paper [24].

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