Interaction of Photosynthetic and Respiratory Electron Transport in Blue-Green Algae: Effect of a Cytochrome *c*-553 Specific Antibody

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An antibody prepared against purified cytochrome *c*-553 from *Nostoc muscorum* inhibits the redox function of cytochrome *c*-553 as checked by a diaphorase assay. 20 to 30% inhibition of NADPH-driven respiratory and light-induced oxygen uptake by *Nostoc* thylakoids is observed when cytochrome *c*-553 specific antibodies were applied. However, only 30 to 50% of the total cytochrome *c*-553 content is released from isolated membrane material, thus being accessible to antibodies. Supplementing the isolated membrane material with excess *Nostoc* cytochrome before adding the antibody abolishes inhibition. The data provide further evidence for soluble cytochrome *c*-553 being a link between photosynthetic and respiratory electron transport in blue-green algae.

Introduction

The interaction of photosynthetic and respiratory electron transport in blue-green algae is of particular interest due to the lack of intracellular compartmentation. As found in various blue-green algae, light affects respiration [1–3], indicative of reducing equivalents originated by (respiratory) dark processes being channeled into the light-driven redox system [4–6].

Some data have been published concerning the dual role of plastoquinone [7] and plastoquinol-cytochrome *f*/*b*-563 reductase [8] in photosynthetic and respiratory electron transport. Evidence is available for cytochrome *c*-553 connecting both redox chains in blue-green algae [9, 10]. Furthermore, it has been demonstrated by cytochrome c_2-specific antibodies that *Rhodopseudomonas capsulata* uses the same cytochrome c_2 in the respiratory and photosynthetic electron-transport chain [11].

These studies were intended to strengthen previous evidence for the dual role of cytochrome *c*-553, by using an antibody against cytochrome *c*-553 as specific inhibitor of cytochrome *c*-553 dependent reactions in photosynthetic and respiratory electron transport of *Nostoc muscorum* thylakoids.

Abbreviations: Chl, chlorophyll *a*; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (buffer, adjusted with NaOH); Methylviologen, N,N′-dimethyl-4,4′-bipyridylium dichloride; TRIS, tris-(hydroxymethyl)aminomethane (buffer, adjusted with HCl).

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Materials and Methods

Nostoc muscorum (ATCC 29151) was grown autotrophically in mineral medium [12] as described previously [4], with potassium nitrate (2 g/l) present in the growth medium. The cells were harvested and resuspended in buffer containing 0.35 M sorbitol, 10 mM $MgCl_2$, 10 mM HEPES, 5 mM Na_2HPO_4/KH_2PO_4, 0.5% bovine-serum albumin, pH 6.9. Thylakoids were prepared at 4 °C by a French pressure cell (600 kg/cm^2, Aminco, Silver Springs, Md, USA; mod. 5-598 AE) yielding a cell homogenate of a final chlorophyll concentration of 0.5 to 1 mg/ml, which was determined according to [13]. Thylakoids were used immediately after preparation because activity decreased to about 70 to 80% after two hours.

Cytochrome *c*-553 from *Nostoc muscorum* (Am. Type Culture Collection, ATCC 29151), *Anabaena variabilis* Kütz. (ATCC 29413), and *Phormidium foveolarum* (1462-1; Algae Culture Collection, University of Göttingen) were purified as described [10]. Cytochrome *c*-553 from *Spirulina platensis* (Nordst.) Gom., strain Leonhard/Wouters (see ref. [14]), was supplied by Prof. H. Böhme of this laboratory, that from the eukaryotic alga *Bumilleriopsis filiformis* Vischer (Xanthophyceae, own stock) was isolated according to [15].

Total cytochrome *c*-553 content was determined by repeated freezing and thawing of the French-press homogenate and resuspended pellets, respectively. After centrifugation, the supernatants were pooled. For estimation of cytochrome *c*-553 remaining in the thylakoid material, an aliquot of the homogenate was centrifuged for 10 min at 12 000 × *g* providing a membrane fraction as green (thylakoid) pellet containing cytochrome *c*-553 and a blue supernatant containing the fraction of cytochrome *c*-553 released from the thylakoids (comp. Table II). For measurement of cytochrome *c*-553 the phycobilins had to be removed according to a modified procedure of [10]: Wetted DEAE-cellulose (about 1 ml, Whatman) was mixed with 1.5 ml of supernatant. After centrifugation, phycobilins were found in the DEAE-pellet, the cytochrome in the supernatant.

An antibody against cytochrome *c*-553 from *Nostoc muscorum* was obtained by injecting 0.5 mg 2 ml of purified cytochrome *c*-553 (the purity was the same as given in [10]), emulsified with 2 ml of complete Freund's adjuvant, into a rabbit's back. The first shot was done subcutaneously. Booster injections were done intramuscular with 0.25 mg of cytochrome *c*-553 every two weeks, and 10 to 20 ml of blood was repeatedly withdrawn from the ear vein. Several antisera were pooled, enriched, and purified by ammonium sulfate precipitation as described [16]. All expts. were done with one preparation. Protein content was estimated according to [17]. Specificity of the antibody was checked by the Ouchterlony double-diffusion test [18].

Enzymatic reduction of cytochrome *c*-553 was performed using a diaphorase system according to [19]. The assay contained, in a final volume of 1 ml, 10 mM TRIS, pH 7.8; 0.1 mM NADP; 3 mM sodium isocitrate; 2 mM $MgCl_2$; 0.5 or 1 μM cytochrome *c*-553 from *Nostoc muscorum;* 4 μM ferredoxin-NADP oxidoreductase (isolated from *Bumilleriopsis filiformis* as described [20]). Oxidation was done with potassium ferricyanide (60 μM cytochrome with 1 mM ferricyanide present; 20 μl of this mixture were used for the 1-ml diaphorase assay). Activity was followed with a Perkin-Elmer Lambda-3 photometer at 553 nm using an extinction coefficient of 25.3 $mM^{-1} \times cm^{-1}$ [21]. The reaction was started by adding isocitrate dehydrogenase and the absorbance change measured after 40 to 50 s, when no further change was observed.

Both NADPH-driven dark respiration and light-induced oxygen uptake were monitored by a Clark-type electrode as described [10].

Bovine-serum albumin, TRIS, HEPES, sodium isocitrate and horse-heart cytochrome 550 were purchased from Sigma, München. Methylviologen was supplied by Serva, Heidelberg, and isocitrate dehydrogenase by Boehringer, Mannheim.

Results

The antibody precipitated purified *Nostoc* cytochrome *c*-553 either in a purified form or when released from *Nostoc* thylakoids as shown in Fig. 1. Only little affinity was found to isolated cytochrome *c*-553 from the blue-green alga *Phormidium foveolarum* and no visible precipitation was detected with horse-heart cytochrome *c*-550 and cytochrome *c*-553 from the blue-green alga *Spirulina platensis* or the (eukaryotic) Xanthophyceae *Bumilleriopsis filiformis.*

To see whether the antibody inhibits the redox function of cytochrome *c*-553, a diaphorase assay was used (Table I). Activity and titer of the antibody fraction were estimated from inhibition of cytochrome *c*-553 reduction. 100 μl of antibody (1 mg protein) completely inhibited the reduction of 0.3 nmol cytochrome *c*-553, while 0.7 nmol cytochrome *c*-553 was inhibited to about 50% only. 200 μl of antibody solution abolished the reduction of 0.7 nmol cytochrome *c*-553. Application of corresponding control sera had little effect. A preincubation time of 15 min was found to be necessary. The control assay showed that the cytochrome *c*-553 applied had not been completely oxidized beforehand by potassium ferricyanide (see Methods).

The influence of the antibody on respiratory and light-induced oxygen uptake is shown in Fig. 2. In a typical assay, the control thylakoids exhibited an

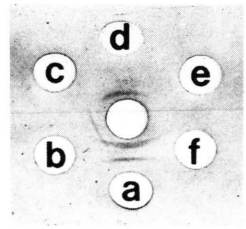

Fig. 1. Ouchterlony double-diffusion test. Center well-purified *Nostoc* cytochrome *c*-553 antibody. Thylakoids from *Nostoc muscorum* (a), isolated cytochromes *c* from: *Nostoc muscorum* (b), horse heart (c), *Phormidium foveolarum* (d), *Bumilleriopsis filiformis* (e), *Spirulina platensis* (f).

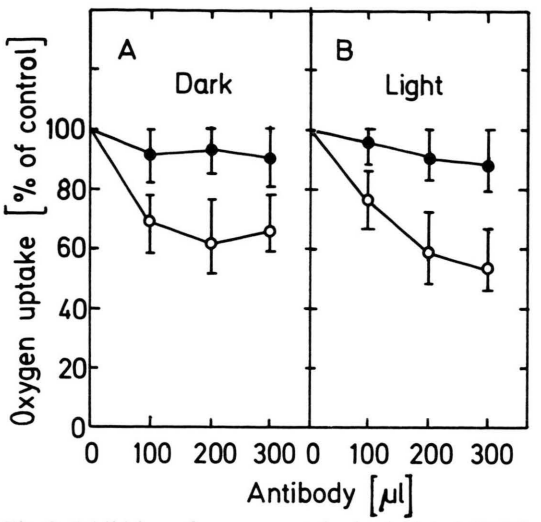

Fig. 2. Inhibition of oxygen uptake by isolated thylakoids from *Nostoc muscorum*, dependent on the amount of antibody (○) and control serum (●) added. Each assay contained 100 µl of thylakoids (equivalent to 80 µg of Chl) and was preincubated in the dark for 15 min with antibody and control serum (10 mg of protein/ml), respectively, before either NADPH was added or the light turned on. (A) NADPH-driven dark respiration, 100% = 7 µmol O_2/mg Chl × h; (B) light-induced oxygen uptake, without methylviologen present, 100% = 53 µmol O_2/mg Chl × h. Each data point is an average obtained from 9 individual experiments.

Table I. Effect of antibody on reduction of *Nostoc* cytochrome *c*-553 in a diaphorase assay. Data were obtained with 0.5 nmol/ml (column 1) and 1 nmol/ml (column 2) cytochrome *c*-553, respectively. Sera contained 10 mg protein/ml. For experimental details see Methods. The average deviation was ± 12% from the means which are given here.

Additions	Reduced cytochrome *c*-553 [nmol/ml]	
	(1)	(2)
(−) Serum (control)	0.30	0.70
+ 100 µl Control serum	0.30	0.60
+ 200 µl Control serum	0.20	0.50
+ 100 µl Antibody serum	0	0.40
+ 200 µl Antibody serum	0	0.08

Table II. Distribution of cytochrome *c*-553 in homogenates of *Nostoc muscorum*. Data from 4 preparations (500 to 800 µg Chl/ml each) are given, obtained from different cell samples. For details to cols. 1 − 3 see Methods.

Experiment No.	Cytochrome *c*-553 [nmol/ml]		
	(1) Homogenate	(2) Supernatant	(3) Pellet
1	0.44	0.20	0.20
2	0.90	0.26	0.53
3	0.83	0.26	0.52
4	0.75	0.32	0.35

NADPH-driven dark respiration of 7 µmol O_2/mg Chl × h, which was partly inhibited by 1 mM KCN; a residual (KCN-insensitive) oxygen uptake of 2.5 µmol O_2/mg Chl × h was observed. The light-induced oxygen uptake using H_2O as electron donor was 53 µmol O_2/mg Chl × h and was increased to 90 µmol O_2/mg Chl × h by the presence of 1 mM methylviologen. The antibody serum inhibited light and dark oxygen uptake to 20 − 30%, while the control serum influenced only slightly (comp. Table I). It should be noted that the degree of inhibition of both dark and light reaction is apparently identical. Again a preincubation time of 15 min was necessary to achieve inhibition. Purification of the antibody serum or extending the preincubation time did not increase the inhibition. Loss of thylakoid activity by dilution processes during preincubation time was negligible.

The amount of cytochrome *c*-553 retained in the isolated membrane material and the fraction released from the thylakoids into the supernatant are listed in Table II. During preparation 50 to 60% of the cytochrome *c*-553 remained bound in the thylakoid pellet, apparently being inaccessible to antibodies. 1 ml of thylakoid suspension equivalent to 500 − 800 µg of chlorophyll released 0.2 to 0.3 nmol/ml cytochrome *c*-553 into the supernatant.

The low inhibition raises the question as to whether the action of the antibody is specific. Therefore, we tried to neutralize the antibody-dependent inhibition by adding cytochrome *c*-553. Inhibition of respiratory and light-induced oxygen uptake was abolished by adding 3 nmol cytochrome *c*-553 to the membranes (= 50 to 80 µg of Chl), before starting preincubation with antibody. We have checked that this amount of cytochrome did not stimulate the rates of control thylakoids. No relief of inhibition was observed, however, when cytochrome *c*-553 was added to antibody-treated thylakoids after a 15-min preincubation time.

Discussion

The specificity observed in the Ouchterlony test would not be sufficient evidence that the antibody may influence the redox function of cytochrome

c-553, because it may bind antigenic determinants which are not functionally important. However, the enzymic reduction of cytochrome *c*-553 was completely inhibited, giving evidence that the antibody applied in this study is directed towards redox function(s) of the cytochrome. The titer was 0.3 nmol cytochrome *c*-553 bound per mg of antibody protein. This antibody to antigen ratio is different from the cell-free system, where 1 mg of antibody protein is needed for a 20 to 30% inhibition. Our explanation is as follows: (a) only 30 to 50% of the total cytochrome *c*-553 is released from the thylakoids, thus being accessible to antibodies, (b) isolated cytochrome *c*-553 molecules are freely accessible, while in the cell-free system some antigenic determinants may be buried in membrane regions out of reach to the antibody, (c) dynamic processes in the membrane may influence antibody-antigen interaction [22] (d) a 3-dimensional connection of antigens, which may be advantageous for inhibition, develops more easily in the *in vitro* system.

Washed membrane preparations exhibit a decreased electron transport due to soluble cytochrome having been removed. It is possible to specifically restore activity by adding back soluble cytochrome *c*-553 [10]. Apparently, both the soluble fraction and the one (still) attached to the thylakoids mediate electron transport. Conceivably, therefore, antibody treatment of our (non-washed) preparations decreases redox activity due to removal of that cytochrome fraction which is present in soluble form (30 to 50% of the total amount). – Some cytochrome *c*-553 may have been lost during application of DEAE-52 cellulose (see Methods). Nevertheless, the relation of total cytochrome *c*-553 to the fraction (still) attached to the thylakoids as well as to the soluble fraction present in the supernatant remained about the same in all four experiments (Table II).

Using antibodies against cytochrome *c*-552 and *Euglena* thylakoids, inhibition of cytochrome photooxidation was found in the presence of cholate only [23]. Apparently, part of the cytochrome *c*-552 is trapped in the membrane. It has been suggested that both membrane sides are exposed, the membrane fraction representing, at least in part, a mixture of closed vesicles and sheets [23, 24]. Probably, changes in membrane orientation had occurred during French-press preparation of thylakoids, allowing partial inhibition. Accordingly, the comparatively low inhibition, which was not increased by excess antibody, indicates localization of a corresponding part of the cytochrome *c*-553 in membrane regions not accessible to the antibody.

In summary, cytochrome *c*-553 acts as a common carrier in photosynthetic and respiratory electron transport: both NADPH-driven dark respiration and light-induced oxygen uptake are inhibited to about the same extent by a cytochrome *c*-553 specific antibody.

Acknowledgements

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