*Biochimica et Biophysica Acta*, 501 (1978) 275—285


BBA 47444

# THE ROLE OF PLASTIDIC CYTOCHROME *c* IN ALGAL ELECTRON TRANSPORT AND PHOTOPHOSPHORYLATION

HERBERT BÖHME, KARL JOSEF KUNERT and PETER BÖGER

*Fachbereich Biologie, Universität Konstanz, D-7750 Konstanz/Giessberg (G.F.R.)*

(Received July 8th, 1977)

## Summary

By an improved isolation procedure chloroplasts could be obtained from the alga *Bumilleriopsis filiformis* (Xanthophyceae) which exhibited high electron transport rates tightly coupled to ATP formation. Uncouplers both stimulate electron transport and inhibit photophosphorylation. These chloroplasts retain almost all soluble cytochrome *c*-553 besides a membrane-bound cytochrome *c*-554.5 (=*f*-554.5). Sonification or iron deficiency removed the soluble cytochrome only with a concurrent decrease of electron transport from water to methyl viologen or to NADP and decreased non-cyclic and cyclic photophosphorylation. However, photosynthetic control and the *P*/2*e* ratios remain unaltered.

In *Bumilleriopsis*, which apparently has no plastocyanin, the soluble cytochrome *c*-553 seemingly links electron transport between the bound cytochrome *c* and *P*-700.

## Introduction

In photosynthesis most of our knowledge of the functional interaction of electron carriers is derived from studies of higher plant chloroplasts. Eukaryotic algae offer many advantages, as they can be cultured easily under controled conditions and allow environmental manipulation. Investigations with mutants, which cannot carry out normal photosynthesis led to a genetic dissection of the photosynthetic electron transport system [1,2].

The major problem with algal material is the isolation of chloroplasts with intact photosynthetic electron transport coupled to phosphorylation. Particularly ATP formation by linear electron transport (e.g. $H_2O \rightarrow$ NADP) could be

Abbreviations: DCMU, 3(3′,4′-dichlorophenyl)-1,1-dimethylurea; *P*-700, pigment 700 (= reaction center chlorophyll of photosystem I); HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; PMS, *N*-methylphenazonium methyl sulfate.

achieved only with isolated chloroplasts of a few species, like e.g. *Bumilleriopsis* [3]. More recently a *Chlamydomonas* system with good photophosphorylation activity has been reported [4,5].

Since the early investigations on algal photosynthesis one major difference to higher plant systems has been recognized, namely the detection of a soluble cytochrome *c*, not present in higher plant chloroplasts [6]. Compared to cytochrome *f* from higher plants its absorbance bands are slightly to the short wavelength side and the molecular weight is typically around 10 000 [7]. It was thought that this soluble *c*-type cytochrome should take over the role of higher plant cytochrome *f*. However, in enzymatic tests or reconstituted photosynthetic systems of higher plant chloroplasts it replaced plastocyanin rather than isolated cytochrome *f* (see refs. 8—10). Moreover, it was noted earlier that aside from the soluble *c*-type cytochrome a membrane-bound species could be detected in algal chloroplast fragments with slightly different spectral properties [11—14]. Since both *c*-type cytochromes were similarly affected in algal mutants, it was concluded that both may represent the same cytochrome, but located in different physical environment [11]. A very recent study succeeded in isolating plastidic bound cytochrome *c* from several algae. The membrane-bound cytochrome *c* differs from the soluble one by its absorption spectrum, molecular weight and kinetic properties in enzymatic tests [15]. The hypothesis was put forward that algal membrane-bound cytochrome *c* should be analogous to cytochrome *f* of higher plants, as proposed already by Grimme and Boardman [14], and the soluble species equivalent to plastocyanin.

On the other hand, with two exceptions known, green algae contain both plastidic soluble cytochrome *c* and plastocyanin and their mutual replacement still has to be investigated. It has been shown for *Bumilleriopsis* that this alga contained no plastocyanin [16] as was briefly reported also for *Euglena gracilis* (ref. 17 and own finding). Especially *Bumilleriopsis* offers the advantage that chloroplasts with high phosphorylation activity in non-cyclic electron transport activities may be isolated [3,18]. Therefore, it appeared to be the appropriate object to investigate the role of soluble cytochrome *c*-553 in photosynthetic electron flow. Furthermore, by our improved isolation procedure chloroplasts were obtained which retain their soluble cytochrome *c*-553 almost completely. Moreover, this chloroplast preparation exhibited high electron transport rates tightly coupled to phosphorylation.

## Materials and Methods

*Growth of algae. Bumilleriopsis filiformis* VISCHER (Xanthophyceae; stock collection of P.B.) was grown under continuous light in a sterile autotrophic medium at 23°C [3,19], the iron complexed with ethylene-diaminetetraacetic acid (EDTA). The iron-deficient cultures contained 1/10 of the iron normally added. Sterile air, enriched with 2% $CO_2$ (v/v) was continuously supplied. Illumination of the algae was provided by fluorescent lamps (Osram 32-1/25-1) with an intensity of 13 000 lux. Cell density at the beginning was $5 \cdot 10^5$—$6 \cdot 10^5$ cells/ml, increasing about 10-fold (7-fold for iron-deficient cultures) within 48 h, when the algae were harvested in the logarithmic phase.

*Isolation of chloroplasts.* The procedure is based on methods for isolation of intact spinach [20] and of type C chloroplasts from *Bumilleriopsis* [21]. The latter method was followed with some modifications. After harvesting, the algal cells were washed once and homogenized with glass beads (1 mm diameter) for 1 s, 10 times at 4000 rev./min with 3-s intervals at half speed (Merkenschlager; Braun, Melsungen) in a medium containing 0.6 M sorbitol, 50 mM 2-(*N*-morpholino)ethanesulfonic acid buffer (adjusted with NaOH), pH 6.2, 1 mM sodium isoascorbate, 3.5% polyvinyl pyrrolidone M 2500, 5 mM $MgCl_2$, and 1% bovine serum albumine. The glass beads were separated from broken cells by filtering through a coarse fritted glass funnel (G1, Schott) into a suction flask. The homogenate (25 ml) was diluted to 70 ml with the homogenization medium and centrifuged 1 min at $1000 \times g$ to remove cell material followed by centrifugation of the supernatant at $3000 \times g$ for 3 min. The chloroplast pellet was suspended in a medium containing: 0.6 M sorbitol, 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (adjusted with NaOH), pH 7.8, 5 mM $MgCl_2$, and 1% serum albumine. Remaining cell material was removed by two successive centrifugations at $1000 \times g$ for 30 s and chloroplasts were obtained from the supernatant by centrifugation at $2000 \times g$ for 2 min. They were resuspended in the medium just mentioned above with a chlorophyll content adjusted to 2 mg/ml. It was found that the high concentration of bovine serum albumine acted beneficially to yield chloroplasts with high cytochrome *c*-553 content [5,22]. Under the phase contrast microscope these chloroplasts appear to be devoid of the outer envelope but otherwise intact.

Sonification of the chloroplast suspension (0.6 mg chlorophyll/ml) was performed with a Branson sonifier (Type B-12, 60 W, power setting at 3) for 10 s at 4°C. After centrifugation at $10\,000 \times g$ for 2 min the pellet was resuspended in the same medium.

Chlorophyll was determined in 80% acetone extracts according to Arnon [23]. This alga only has chlorophyll *a*.

Light-induced oxygen uptake or evolution was measured with a YSI-oxygen electrode (Mod. 53) at 25°C. Saturating red light (Schott RG 610, 3 mm, and Filtraflex heat filters, Balzers) was provided by a 100 W tungsten iodine lamp with an intensitivity of 600 $J/m^2$ per s. The reaction mixture contained in a 3 ml final volume: 0.6 M sorbitol, 5 mM $MgCl_2$, 50 mM HEPES buffer, pH 7.8, and 10 mM $K_2HPO_4$.

Photophosphorylation was measured by incorporation of $^{32}P$ into ATP according to Avron [24], see also ref. 18. Open cuvettes containing 1 ml of the reaction mixture (minus phosphate) and chloroplasts equivalent to 10 $\mu$g of chlorophyll were illuminated for 2—3 min at 25°C with the electron acceptors as indicated in Tables II and III; saturating white light (with Filtraflex heat filters) was 200 $J/m^2$ per s.

Cytochrome *c*-553/554.5 content retained in the photosynthetic membrane was determined by difference absorption spectra (mod. DW-2 of Aminco, Silver Spring, Md., U.S.A.) of a chloroplast suspension treated with 0.1% Triton X-100 [25]. Triton X-100 converts cytochrome *b*-559, also present in *Bumilleriopsis* chloroplasts, to its low-potential form facilitating the determination of both *c*-type cytochromes. A line was drawn between the isosbestic points at

541 and 561 nm and the cytochrome concentration was estimated from the absorbance difference to the peak maximum using a $\Delta\epsilon$ of 16.8 $mM^{-1} \cdot cm^{-1}$ [26]. Since the molar extinction coefficient of membrane-bound cytochrome *c*-554.5 is not known, no further corrections were made. By repeated determinations the $\alpha$-band was found between 554 and 555 nm. The fraction of soluble plastidic cytochrome *c* was determined in the supernatant of chloroplast material after weak sonification (see above).

The content of pigment 700 (*P*-700) was estimated from potassium ferricyanide-oxidized minus ascorbate-reduced difference spectra using a $\Delta\epsilon_{700\,nm}$ = 64 $mM^{-1} \cdot cm^{-1}$ [27]. *Bumilleriopsis* ferredoxin was prepared according to ref. 28.

## Results

Chloroplasts from the alga *B. filiformis* can be prepared which retain most of their so-called soluble cytochrome *c*-553 (Table I). Upon short-term sonification of these chloroplasts soluble cytochrome *c*-553 is almost completely lost and can be recovered quantitatively from the supernatant. The sonified chloroplasts still contain a thylakoid-bound *c*-type cytochrome. This results in a peak shift of the $\alpha$-band of the reduced form from 553 to 554.5 nm (Fig. 1), pointing to the presence of two difference cytochromes in these chloroplasts. In accordance with the findings of Boardman's group [14] with *Chlorella* and Wood [15] with other algal species the membrane-bound fraction with an $\alpha$-peak at 554.5 nm may be analogous to cytochrome *f* of higher plants. In the course of these experiments it was noted that the content of soluble cytochrome *c*-553 in cells and chloroplasts was variable depending on the culture conditions. This has already been noted for other algae as well [11,12,29]. Maximal values of four molecules of cytochrome *c*-553 and about one cytochrome *c*-554.5 per one molecule of *P*-700, the Photosystem I reaction center chlorophyll, could be measured (see Table I).

The data of Table II show that these chloroplasts exhibit high rates of electron transport with either methyl viologen or NADP as terminal acceptors.

TABLE I

CONTENT OF CYTOCHROME *c*-553/554.5 IN *BUMILLERIOPSIS FILIFORMIS*

| | nmol cytochrome *c* per $\mu$mol chlorophyll | Cytochrome/*P*-700 (nearest integer) |
|---|---|---|
| Normal culture | | |
| Whole cells * | 5.5—6.5 | 4 |
| Chloroplasts | 4 —5 | 3 |
| Sonified chloroplasts | 1.4—1.5 | 1 ** |
| Iron-deficient culture | | |
| Whole cells | 3.3—3.7 | 2 |
| Chloroplasts | 2.5—2.8 | 2 |

* Maximal value obtained was 8.3 nmol cytochrome *c* per $\mu$mol chlorophyll, approximately a total cytochrome *c* per *P*-700 ratio of (mol/mol).

**This cytochrome is bound cytochrome *c*-554.5.

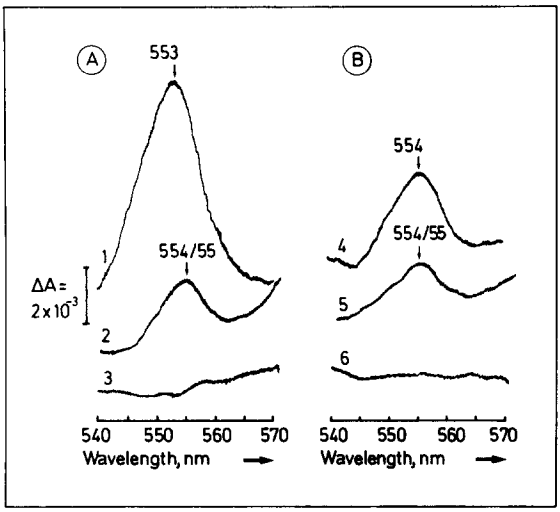


Fig. 1. Reduced minus oxidized difference spectra of Triton-treated chloroplasts isolated from (A) normal and (B) iron-deficient cultures of *B. filiformis* (see Materials and Methods; hydroquinone minus potassium ferricyanide, approx. 0.1 mM). (A) 1, control chloroplasts and 2, sonified chloroplasts: 66 μg chlorophyll/ml. A cytochrome *c*/chlorophyll ratio of 1 : 205 (curve 1) and 1 : 640 (curve 2) can be calculated. (B) 4, control chloroplasts = 62 μg chlorophyll/ml; 5, sonified chloroplasts = 57 μg chlorophyll/ml. From the curves obtained with chloroplasts from an iron-deficient culture a cytochrome *c*/chlorophyll ratio of (4) 1 : 432 and (5) 1 : 670 can be calculated. 3 and 6, baseline recorded at the same sensitivity; optical bandpass = 2 nm.

Interestingly these chloroplasts also showed photosynthetic control which means that the basal rate (minus ADP) could be stimulated by a factor of 2—3 upon addition of ADP. By addition of uncouplers like $NH_4Cl$ or gramicidin a stimulation by a factor of 4 was observed. As expected, both uncouplers inhibit phosphorylation completely (Table IIIA), which was not observed by others [4,5]. The coupling phenomenon allows one to measure the ADP/O ratios [30] as demonstrated in Fig. 2, hence calculating the *P*/2*e* ratio by state 3/state 4 transitions (terminology adopted from Chance and Williams [31]). Routinely a *P*/2*e* ratio of one could be obtained, the basal rate was not subtracted (see ref. 30) (Table II). This value was independently confirmed by measuring $O_2$ evolution and $^{32}P$ incorporation into ADP under the same experimental conditions (data not shown). It should be noted that addition of ATP (0.1–1 mM) did not affect the basal electron transport, as was reported for spinach chloroplasts.

Sonification of chloroplasts, thereby removing soluble cytochrome *c-553*, resulted in an almost complete inhibition of electron transport and phosphorylation. Nevertheless the residual rate of electron transport could still be stimulated by uncoupler. The partial Photosystem II reaction from $H_2O$ to diaminodurene remained unimpaired (Table II). Note that the content of membrane-bound cytochrome *c*-554.5 per *P*-700 remained unaltered under these conditions (Fig. 2). This suggests that cytochrome *c*-553 mediates electron flow between cytochrome *c*-554.5 and *P*-700.

In contrast to earlier findings with *Euglena* chloroplasts [17] electron transport could not be restored by addition of cytochrome *c*-553 to sonified *Bumil-*

TABLE II

ELECTRON TRANSPORT RATES AND COUPLING IN ISOLATED *BUMILLERIOPSIS FILIFORMIS* CHLOROPLASTS

The minus sign indicates oxygen uptake, the other rates represent oxygen evolution; methyl viologen is 1,1'-dimethyl-4,4'-dipyridylium dichloride; diaminodurene is 2,3,5,6-tetramethyl-*p*-phenylenediamine.

| | Total cytochrome *c* / Chlorophyll | Electron transport system | Rates in $\mu$mol $O_2$/mg chlorophyll per h | | | |
|---|---|---|---|---|---|---|
| | | | (—)ADP | (+)ADP | (+)$NH_4Cl$ | ADP/O ratio |
| Chloroplasts from normal culture | 1 : 250 | $H_2O \rightarrow$ methyl viologen | —51 | —104 | —199 | 0.93 |
| | | $H_2O \rightarrow$ NADP | 36.6 | 95 | 130 | 1.02 |
| Chloroplasts from iron-deficient culture | 1 : 450 | $H_2O \rightarrow$ methyl viologen | —38 | —81.5 | —95 | 1.2 |
| | | $H_2O \rightarrow$ NADP | 22 | 54 | 46 | 1.1 |
| | 1 : 540 | $H_2O \rightarrow$ methyl viologen | —27 | —54 | —78 | (—) |
| Sonified chloroplasts from normal culture | 1 : 650 | $H_2O \rightarrow$ methyl viologen | —6 | —12 | —16 | (—) |
| | | $H_2O \rightarrow$ methyl viologen + cytochrome *c*-553 (1 $\mu$M), soluble | —6 | (—) | —16.5 | (—) |
| | | $H_2O \rightarrow$ diaminodurene * | 200 | (—) | (—) | (—) |
| Sonified chloroplasts with 70 $\mu$M soluble cytochrome *c*-553 added | not measured | $H_2O \rightarrow$ methyl viologen | —27.5 | (—) | —27.5 | (—) |

* Electron transport rate with oxidized diaminodurene as acceptor in control chloroplasts was 230 $\mu$mol $O_2$/mg chlorophyll per h. Conditions as in Fig. 2.

TABLE III

(A) PHOSPHORYLATION WITH *BUMILLERIOPSIS FILIFORMIS* CHLOROPLASTS *

Methyl viologen, 0.1 mM; NADP, 0.5 mM; ferredoxin, 10 nmol/ml; 5-methylphenazonium methyl sulfate, 0.015 mM.

| | Electron transport system | Additions | μmol ATP formed/ mg chlorophyll per h |
|---|---|---|---|
| Chloroplasts from normal culture * | $H_2O \rightarrow$ methyl viologen | — | 208 |
| | | + 5 μg gramicidin | 19 |
| | | + 2.5 mM $NH_4Cl$ | 36.5 |
| | $H_2O \rightarrow$ NADP | — | 196 |
| | | + 5 μg gramicidin | 9 |
| | | + 2.5 mM $NH_4Cl$ | 22.5 |
| | 5-methylphenazonium methyl sulfate | — | 183 |
| | | + 1 μM DCMU | 43 |
| | | + 1 mM ascorbate | 211 |
| | | + 1 mM ascorbate/1 μM DCMU | 178 |
| Chloroplasts from iron-deficient culture | $H_2O \rightarrow$ methyl viologen | — | 155 |
| | $H_2O \rightarrow$ NADP | — | 151.5 |
| | 5-methylphenazonium methyl sulfate | + 1 mM ascorbate | 171 |
| Sonified chloroplasts from normal culture | $H_2O \rightarrow$ methyl viologen | | 15 |
| | $H_2O \rightarrow$ NADP | | 13.5 |
| | 5-methylphenazonium methyl sulfate | + 1 mM ascorbate | 15.0 |

* Cytochrome/chlorophyll ratios are comparable to the values of Table II.

(B) EFFECT OF CYTOCHROME *c*-553 ON PHOSPHORYLATION OF SONIFIED *BUMILLERIOPSIS FILIFORMIS* CHLOROPLASTS

Conditions as in A.

| | Electron transport system | Additions | μmol ATP formed/ mg chlorophyll per h |
|---|---|---|---|
| Sonified chloroplasts | $H_2O \rightarrow$ methyl viologen | — | 6.5 |
| | | + 1 μM cytochrome *c*-553, soluble | 6.0 |
| | | + 1 mM ascorbate | 9.0 |
| | | + 1 μM cytochrome *c*-553, soluble + 1 mM ascorbate | 9.5 |
| Sonification in presence of 70 μM added soluble cytochrome *c*-553 | $H_2O \rightarrow$ methyl viologen | — | 8.0 |
| | | + 1 mM ascorbate | 16.0 |

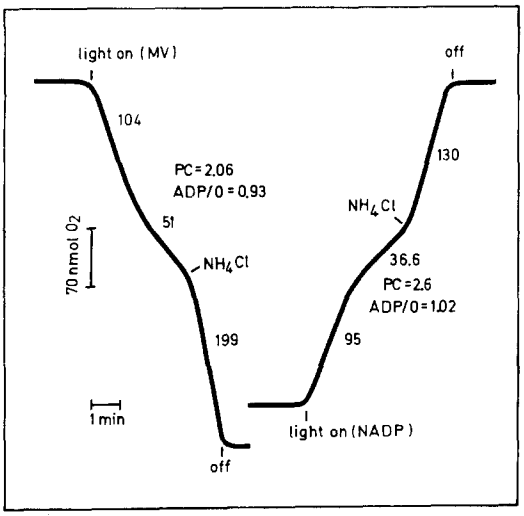


Fig. 2. Photosynthetic oxygen uptake ($H_2O \rightarrow$ methyl viologen, MV) or evolution ($H_2O \rightarrow$ NADP) with *Bumilleriopsis* chloroplasts. 300 nmol ADP was added before the light was switched on. This amount of ADP was consumed (i.e. transformed into ATP) while electron transport proceeded as indicated by the declining rate during the second half of the reaction. Additions as indicated: $NH_4Cl$ = 2 mM; methyl viologen = 0.1 mM; NADP = 0.6 mM with 6.6 $\mu$M ferredoxin. Numbers along the traces indicate $\mu$mol $O_2$/ml chlorophyll per h. PC, photosynthetic control ratio.

*leriopsis* chloroplasts (Table II). Some protection of electron transport activity by addition of cytochrome *c*-553 (70 $\mu$M) during sonification was observed. The absolute electron transport rate, however, did not exceed 20% of the electron transport of control chloroplasts.

One objection may be made against this kind of experiments, namely that sonification may alter the membrane structure or may solubilize additional components other than cytochrome *c*-553, and thereby be inhibitory to coupled electron flow. We tried, therefore, to manipulate the soluble cytochrome *c*-553 content by varying the culture conditions. It was found that iron deficiency in the growth medium somewhat affected the growth rates and the chlorophyll content but more severely the cytochrome *c*-553 content (Bohner, H., unpublished experiments). The total cytochrome *c*-553 content in chloroplasts isolated from iron-deficient cells dropped to about 1/3—1/2 of the control value (compare Table I). As shown in Fig. 1, the ratio of bound cytochrome *c*-553 per *P*-700 of 1 was not influenced. The data of Table II show clearly that with decreasing amounts of cytochrome *c*-553 the rate of electron transport decreased simultaneously. Interestingly the coupling and the *P*/2*e* ratio remained unaltered. It appears that cytochrome *c*-553 is an obligatory electron transport mediator but not directly involved in the energy-conserving mechanism.

In Table IIIA the rates of non-cyclic ATP formation obtained with *Bumilleriopsis* chloroplasts are shown. Comparing the phosphorylation activity obtained with normal, iron-deficient and sonicated chloroplasts it is obvious that ATP formation decreases with decreasing cytochrome *c*-553 content. Surprisingly, cyclic phosphorylation catalyzed by *N*-methylphenazonium methyl sulfate (PMS) seems to require cytochrome *c*-553 as well. Increasing the concen-

tration of this cofactor up to an 8-fold excess of the optimal concentration did not overcome this inhibition by absence of cytochrome *c*-553 in sonified chloroplasts.

Re-addition of isolated cytochrome *c*-553 to sonified chloroplasts also did not stimulate phosphorylation activity (see Table IIIB). When cytochrome *c*-553 was present during sonification some protection of cyclic and non-cyclic phosphorylation was observed; however, the rates did not exceed 10% of the control (compare ref. 33). As found by others DCMU inhibits the PMS-catalyzed phosphorylation. This inhibition is prevented by ascorbate acting as a quencher for superoxide, thereby preventing the photoconversion of the added catalyst to pyocyanin which does not catalyze cyclic phosphorylation under aerobic conditions in the presence of DCMU [5].

## Discussion

The results presented above demonstrate that chloroplasts can be isolated from the alga *B. filiformis* with high rates of both cyclic and non-cyclic electron transport coupled to phosphorylation (compare also ref. 18). Control ratios of 2—3 upon ADP addition and about 4 upon addition of uncouplers could be obtained (Fig. 2). This is in contrast to reports on phosphorylating algal fragments where neither ADP nor uncouplers stimulated electron transport [4,5,32]. Since methylamine did not inhibit photophosphorylation in algal fragments it was concluded that the membrane potential is the main driving force for ATP formation [4,5]. Algal chloroplasts used in this study, however, exhibit properties similar to higher plant chloroplasts, i.e. uncoupling by either $NH_4Cl$ or gramicidin stimulates electron flow and inhibits phosphorylation.

A *P*/2*e* ratio around 1.0 was routinely measured; maximal values of 1.2 were reached. As shown in Results these chloroplasts retain most of the so-called soluble cytochrome *c*-553. The approximate molar ratios of P-700, bound cytochrome *c*-554.5 and soluble cytochrome *c*-553 were 1 : 1 : 3—4, respectively. The obligatory requirement for cytochrome *c*-553 in electron transport was shown by comparing electron transport and phosphorylation rates of control chloroplasts with the rates obtained after removing cytochrome *c*-553 by sonification. Although this procedure does not influence the lamellar cytochrome *c*-554.5 content and liberates cytochrome *c*-553 almost completely, cyclic and non-cyclic electron transport rates coupled to phosphorylation are substantially lowered. A correlation of PMS-catalyzed cyclic phosphorylation and cytochrome *c*-552 content has been observed with *Euglena* chloroplasts, but the presence of two *c*-type chromosomes with different functions was not recognized [33]. In chloroplasts isolated from iron-deficient cultures where the content of soluble but not lamellar bound cytochrome *c* was decreased by a physiological parameter, cyclic and non-cyclic electron transport and phosphorylation were almost equally affected. Interestingly, the photosynthetic control and *P*/2*e* ratios remain unaltered suggesting a direct function of "soluble" cytochrome *c*-553 in electron transport mediation but not in energy conservation.

It has been shown before that in the alga *Bumilleriopsis* plastocyanin is ab-

sent and cytochrome *c*-553 may be directly oxidized by Photosystem I [16].

Similar to the data obtained with soluble cytochrome *c*-552 from *Euglena* [8,33] cytochrome *c*-553 rather behaved as a substitute for plastocyanin than for cytochrome *f* of higher plants in a variety of enzymatic tests and photoreactions [16] (compare also refs. 15 and 34).

Both cytochrome *c*-553 and plastocyanin (of higher plants) are acidic proteins of low molecular weight. They appear to be peripherally located in the membrane as they are easily removed by physical treatments like freeze-thawing, French-press or sonification. A similar pool size of 3—4 molecules per reaction center I for cytochrome *c*-553 (*Bumilleriopsis*) and plastocyanin (higher plants) is observed [35]. Both proteins seem to interact directly with *P*-700, the reaction center chlorophyll [16]. It appears that comparable to cytochrome *f* of higher plants the membrane-bound cytochrome *c*-554.5 mediates electron transport between the lipophilic quinone pool and extrinsic cytochrome *c*-553 [15]. An obligatory sequential electron transfer between cytochrome *c*-554.5 → cytochrome *c*-553 → *P*-700 is not proven by the experiments presented, but it is compatible with our data.

## Acknowledgements

These investigations were supported by the Deutsche Forschungsgemeinschaft through its Sonderforschungsbereich No. 138. We thank H. Bohner for helping us with experimental experience on algae cultures and their physiology.

## References

1 Levine, R.P. (1968) Science 162, 768—771
2 Bishop, N.I. (1971) Methods Enzymol. 23, 130—143
3 Böger, P. (1969) Z. Pflanzenphysiol. 61, 85—97
4 Brand, J.J., Curtis, V.A., Togasaki, R.K. and San Pietro, A. (1975) Plant Physiol. 55, 187—191
5 Yannai, Y., Epel, B.L. and Neuman, J. (1976) Plant Sci. Lett. 7, 295—304
6 Lemberg, R. and Barrett, J. (1973) The Cytochromes, Academic Press, New York
7 Yakushiji, E. (1971) Methods Enzymol. 23, 364—368
8 Elstner, E., Pistorius, E., Böger, P. and Trebst, A. (1968) Planta 79, 146—161
9 Hauska, G., McCarty, R.E., Berzborn, R.J. and Racker, E. (1971) J. Biol. Chem. 246, 3524—3531
10 Nelson, N. and Racker, E. (1972) J. Biol. Chem. 247, 3848—3853
11 Gorman, D.S. and Levine, R.P. (1966) Plant Physiol. 41, 1648—1656
12 Biggins, J. (1967) Plant Physiol. 42, 1447—1456
13 Powls, R. Wong, J. and Bishop, N.I. (1969) Biochim. Biophys. Acta 180, 490—499
14 Grimme, L.H. and Boardman, N.K. (1974) in Proceedings of the 3rd International Congress of Photosynthesis (Avron, M., ed.), pp. 2115—2124, Elsevier, Amsterdam
15 Wood, P.M. (1977) Eur. J. Biochem. 72, 605—612
16 Kunert, K.J. and Böger, P. (1975) Z. Naturforsch. 30c, 190—200
17 Katoh, S. and San Pietro, A. (1967) Arch. Biochem. Biophys. 118, 488—496
18 Böger, P. (1971) Methods Enzymol. 23A, 242—248
19 Hesse, M. (1974) Planta 120, 135—146
20 Jensen, R.G. and Bassham, J.A. (1966) Proc. Natl. Acad. Sci. U.S. 56, 1095—1101
21 Spiller, H. and Böger, P. (1977) Photochem. Photobiol. 26, 397—402
22 Wallach, D., Bar Nun, S. and Ohad, I. (1972) Biochim. Biophys. Acta 267, 125—137
23 Arnon, D.I. (1949) Plant Physiol. 24, 1—5
24 Avron, M. (1960) Biochim. Biophys. Acta 40, 257—272
25 Böhme, H. (1976) Z. Naturforsch. 31c, 68—77
26 Lach, H.-J., Ruppel, H.G. and Böger, P. (1973) Pflanzenphysiol. 70, 432—451
27 Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 160—171
28 Böger, P. (1969) Z. Pflanzenphysiol. 61, 447—461

29 Böger, P. and San Pietro, A. (1967) Z. Pflanzenphysiol. 58, 70—75
30 Hall, D.O. (1976) in The Intact Chloroplast (Barber, J., ed.), pp. 135—170, Elsevier, Amsterdam
31 Chance, B. and Williams, G.R. (1955) Nature 176, 250—254
32 Satoh, V.L., Levine, R.P. and Neumann, J. (1971) Biochim. Biophys. Acta 253, 437—448
33 Wildner, G.F. and Hauska, G. (1974) Arch. Biochem. Biophys. 164, 127—135
34 Kunert, K.J., Böhme, H. and Böger, P. (1976) Biochim. Biophys. Acta 449, 541—553
35 Böhme, H. (1977) Eur. J. Biochem., in the press