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A MUTANT STRAIN OF *SCENEDESMUS OBLIQUUS* DEFICIENT IN RIBULOSE DIPHOSPHATE CARBOXYLASE, CYTOCHROME *f* AND PHOTOSYSTEM II ACTIVITY

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SUMMARY

The partial reactions of photosynthesis shown by strain F208, a non-photosynthetic mutant strain of *Scenedesmus obliquus*, have been compared with those performed by other mutant strains which lacked; Photosystem II activity (strains 11 and F131), cytochrome *f* (strain 50), *P*-700 and cytochrome *f* (strain F119), and *P*-700 (strains F139 and 199). In this respect the properties of strain F208 were those that would be expected if Photosystem II activity and cytochrome *f* were not present in this strain. Examination of the composition of strain F208 has shown the absence of cytochrome *f* in both the soluble and the membrane-bound form. The considerably lower level of plastoquinone compared to that found in the wild type is characteristic of the strains which lack Photosystem II activities.

Fraction 1 protein could not be detected in extracts of strain F208 by sedimentation velocity experiments in the ultracentrifuge, and only 7 % of the wild type ribulose diphosphate carboxylase activity was found after chromatography of these extracts on DEAE-cellulose.

The properties of strain F208 are compared with those of the ac-20 and cr-1 strains of *Chlamydomonas rheinhardi*, both of which have a deficiency of ribulose diphosphate carboxylase which is considered to result from a deficiency of chloroplast ribosomes. Strain F208 resembles these strains in its abnormal chloroplast ultrastructure and its decreased levels of the RNA forms derived from the chloroplast ribosomes when compared with the wild type.

Chloroplast fragments isolated from strains of *S. obliquus* which lacked cytochrome *f* (strains 50 and F208) were able to use diaminodurene and ascorbate as an electron donor to Photosystem I. Since this reaction was inhibited by mercuric salts it would appear that plastocyanin, but not cytochrome *f*, was involved in this electron transfer.

Abbreviations: Cl_2-indophenol, 2,6-dichloroindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea.

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INTRODUCTION

As in other biochemical fields, major contributions to the understanding of photosynthesis have been made with the aid of mutant organisms in which the process has been disrupted.

Such work has utilised two organisms, *Chlamydomonas rheinhardi* [1, 2] and *Scenedesmus obliquus* [3]. It has proved possible to isolate mutant algal strains blocked in each of the important areas of photosynthesis; photosynthetic electron transfer, photophosphorylation and reactions of carbon dioxide fixation. Mutant strains blocked in photosynthetic electron transfer reactions are now very numerous. Bishop [4] originally classified this type of mutant into two groups: those unable to perform a Hill reaction (oxygen mutants) and those which did not show any photoreduction (carbon dioxide mutants). More recently these have been referred to as Photosystem II and Photosystem I mutants respectively [5]. A mutant strain of *C. rheinhardi* blocked in the terminal stages of ATP synthesis has been isolated [6]. This strain was unable to couple electron transfer to phosphorylation, although electron transfer itself and light-dependent pH changes were unaffected. Surprisingly, only a single algal strain specifically blocked in one of the reactions of carbon dioxide fixation is known [7]. This strain of *C. rheinhardi* is deficient in phosphoribulokinase activity. Another mutant, *C. rheinhardi* ac-20 [8–10], is unable to synthesise cytochrome *b*-559 and ribulose diphosphate carboxylase. These lesions are considered to arise from the inability of this strain to form chloroplast ribosomes, which have been suggested to be the site of the synthesis of the large subunit of ribulose diphosphate carboxylase. We now report the isolation and characterisation of a mutant strain of *Scenedesmus obliquus* which is also unable to synthesise a normal amount of ribulose diphosphate carboxylase.

METHODS AND MATERIALS

Culture of algal strains

S. obliquus (strain D_3) was cultured heterotrophically at 28 °C on nitrate medium supplemented with 0.5 % glucose and 0.25 % yeast extract [11]. Mutant strains 11 and 50, derived from the wild type by X-radiation and selected because of their inability to assimilate $^{14}CO_2$ were obtained from Dr. N. I. Bishop. Mutant strains F119, F131, F139, F199 and F208 were derived from the wild type by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and selected as strains showing high levels of chlorophyll fluorescence [12].

Whole cell photoreactions

Photosynthesis was measured as the rate of fixation of $^{14}CO_2$. Flasks containing in a total volume of 1 ml, 20 μl packed cell volume of algal cells, 30 μmol KPO_4 buffer pH 7.5, 10 μmol $NaH^{14}CO_3$ (0.5 Ci/mol) were incubated at 28 °C for 10 min in an illuminated (12 000 lux, white light) Gilson differential respirometer. The reaction was terminated by the addition of 0.1 ml of formic acid. 0.1-ml aliquots were placed on planchets and radioactivity measured with a Nuclear Chicago Gas flow counter.

p-Benzoquinone-Hill reaction rates were measured as described by Bishop [4],

light-dependent anaerobic glucose uptake and photoreduction as described by Pratt and Bishop [13] a Braun photosynthetic Warburg apparatus was used for photoreduction.

Chloroplast photoreactions

Algal chloroplast fragments were prepared as described by Bishop [3], except that the cells were broken by shaking with 0.3 mm diameter glass beads (20 ml cell suspension : 20 g beads) for 1 min at 4000 rev./min in a Braun cell homogeniser, liquid CO_2 being used as a coolant.

The ability of the chloroplast fragments to photoreduce various electron acceptors was measured at room temperature using a Zeiss PMQ II spectrophotometer which had been modified to allow continuous measurement of the photoreactions. The actinic light from a 70 watt tungsten filament lamp was passed through Kodak-Wratten filters, 29 (red) and HRl (infra-red cut out), and focussed on the top of the cuvette containing the reaction mixture. A guard filter was placed between the cuvette and the photomultiplier to cut out any scattered light. The reaction mixture used for $NADP^+$ photoreduction was chloroplast fragments (50 μg chlorophyll), saturating ferredoxin and ferredoxin-$NADP^+$ reductase, 1 μmol $NADP^+$, 3 μmol KCN, 5 μmol $MgCl_2$, 150 μmol tricine pH 7.5, 90 μmol KCl in 3 ml. Guard filter was Kodak-Wratten 18A. When Cl_2-indophenol/ascorbate was used as electron donor, 0.12 μmol Cl_2-indophenol, 10 μmol sodium ascorbate and DCMU to a final concentration of $2 \cdot 10^{-6}$ M were added.

For ferricyanide photoreduction, 2 μmol of $K_3Fe(CN)_6$ were added in place of $NADP^+$. Guard filter was Kodak-Wratten 47B.

Phenazine methosulphate catalysed photophosphorylation was measured as described by Pratt and Bishop [13]. Methyl viologen photoreduction was determined as described by Trebst and Pistorious [14].

Fractionation of algal cells

A method (summarised in Fig. 1) was developed from that of Powls et al. [5] to identify the photosynthetic electron transfer chain components present in the algal strains. Algal cells were broken by grinding with glass beads (80 ml packed cell volume of algae : 120 ml 0.4 M Tris/HCl pH 7.5 : 170 ml 0.3 mm diameter glass beads) in the Dynomil (Willy A. Bachofen, Basle) at a blade speed of 2 m/s. Cooling was achieved by continually circulating the breaking chamber with a salt/ice mixture at -5 °C. A total breaking time of 10 min was used, 2-min bursts separated by 1-min intervals. The beads were removed from the broken cell extract by passage through a sintered-glass funnel. Proteins were removed from the extract by differential ammonium sulphate precipitation and separated by chromatography on substituted celluloses. A portion of the red brown protein solution prior to adsorption on to DEAE-cellulose was used to look for fraction 1 protein, which has very characteristic sedimentation properties, in the Beckman model E analytical ultracentrifuge.

P-700 was detected by its chemically induced difference spectrum on *P*-700-enriched chloroplast fragments prepared as described by Kok [15] from the 40 000 $\times g$ residue of centrifugation of the broken cell fragments prior to ammonium sulphate precipitation.

A solution containing the chloroplast membrane-bound cytochromes was

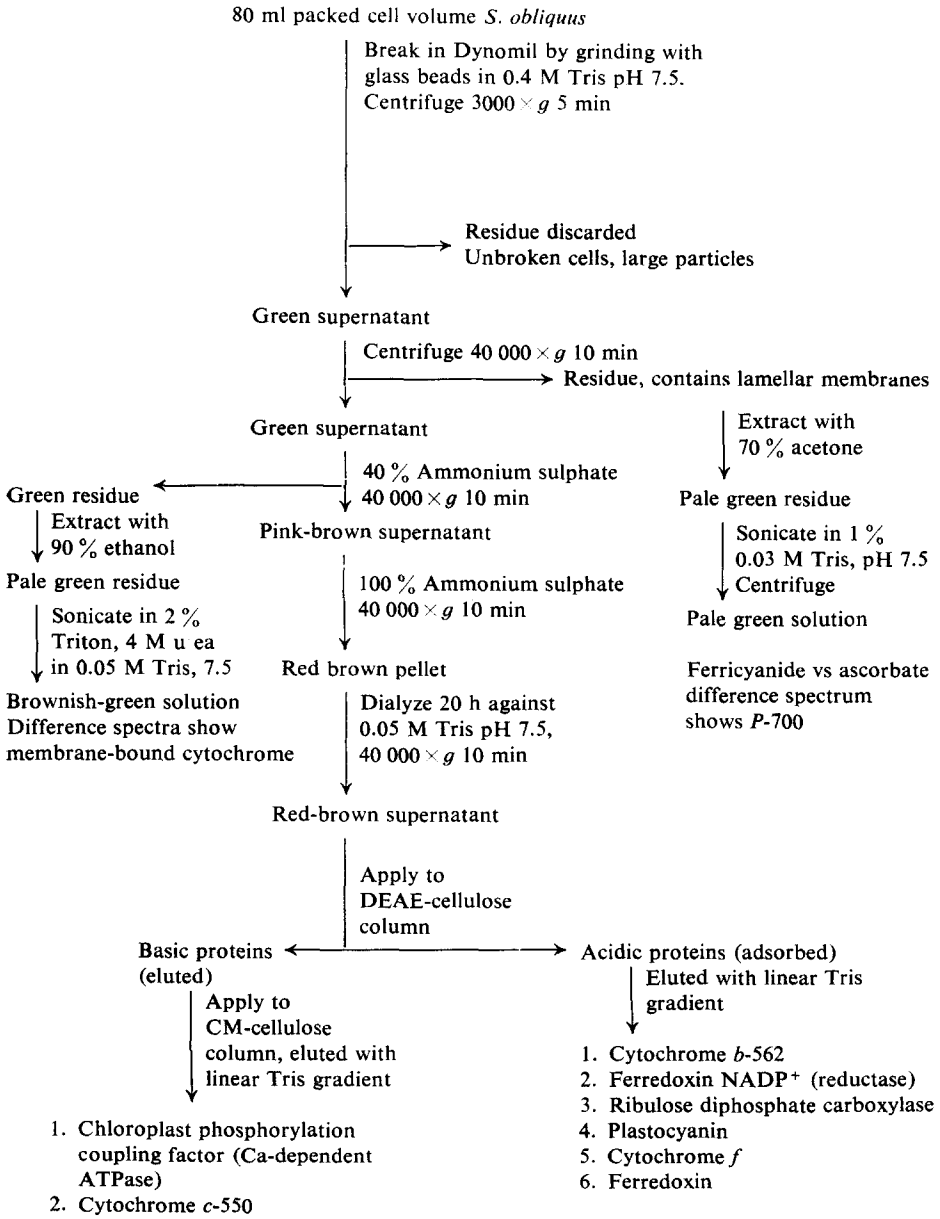

Fig. 1. Scheme for the fractionation of algal cells into their proteinaceous components.

obtained by treatment of the 40 % saturated ammonium sulphate precipitate as described by Garewal et al. [16]. Chemically induced difference spectra of this solution enabled its cytochrome components to be identified.

Quinones were estimated after separation by chromatography of the algal lipid extracts. The algae from 31 of culture were extracted with hot methanol (about 400 ml)

until all the chlorophyll had been removed from the cells. The extracted lipid was transferred to light petroleum (B. P. 40–60 °C) by adding 400 ml to the methanol extract followed by 1 litre of saturated NaCl solution. The petrol extract was washed twice with further salt solution, dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo. Approx. 500 mg of the lipid extract was applied to a 1.5×15 cm column of acid-washed alumina (Woelm) deactivated to Brockman Grade 3. Lipids were eluted with increasing concentrations of diethyl ether in light petroleum (ether/petroleum). Eluting solvents were 0.5 %, 2 %, 6 % and 10 % ether/petroleum. β-carotene was eluted by 0.5 % ether/petroleum, plastoquinone by 2 % and ubiquinone by 6 % ether/petroleum. The eluted fractions were evaporated to dryness, quinone concentrations were determined from the difference in absorption of the ethanolic solutions after reduction with $NaBH_4$. The extinction coefficients used were $\Delta\varepsilon_{275nm}$ 12 250 for ubiquinone and $\Delta\varepsilon_{255nm}$ 17 800 for plastoquinone.

Fractionation of algal RNA species was achieved using a modification of the method of Loening [17]. Algal cells were broken by shaking with glass beads in a Braun cell homogeniser at 4000 rev./min for 3 min (3 ml packed cell vol. of algal cells : 20 ml 0.5 % dodecyl sulphate in 0.5 M Tris/HCl pH 7.5 : 20 g 0.3 mm diameter glass beads). After centrifugation of the homogenate at $1000 \times g$ for 10 min, the supernatant was stirred for 30 min with 20 ml of an aqueous solution containing 200 g of phenol, 28 g *m*-cresol and 0.2 g of 8-hydroxyquinoline [18]; the upper aqueous phase which separated on centrifugation at $12\,000 \times g$ for 15 min contained the nucleic acid and 20 μl aliquots were applied to 2.5 % polyacrylamide gels. After electrophoresis the gels were stained in a 1 % aqueous solution of methylene blue and destained in water after which they were scanned at 600 nm with a Gilford 240 spectrophotometer equipped with a linear transport attachment.

Chlorophyll · protein complexes derived from the two photosystems were separated as described by Gregory et al. [19].

Ribulose diphosphate carboxylase was assayed as described by Paulsen and Lane [20], phosphoribulokinase as described by Moll and Levine [7], ferredoxin, ferredoxin-$NADP^+$ reductase, plastocyanin and cytochrome *f* as previously described [5].

Chloroplast structure

Heterotrophically grown algal cells were fixed in glutaraldehyde and osmium tetroxide, embedded in Epon and post-stained with lead citrate. Micrographs were obtained using a JEOL JEM J7 electron microscope.

RESULTS AND DISCUSSION

The ability of the mutant strains to perform the various whole cell photoreactions is summarised in Table I. Photosystem I has been shown to mediate both the light-dependent uptake of glucose [21], and also the fixation of CO_2 by photoreduction under H_2 [22], whereas Photosystem II alone is required for the Hill reaction using *p*-benzoquinone as electron acceptor [4]. Consequently, these strains can be classified as Photosystem I or Photosystem II mutants based on their ability to carry out these photoreactions. Mutant strains 11 and F131 are blocked in Photosystem II activity whereas strains 50, F119, F139 and F199 are unable to perform the Photo-

system I-mediated reactions. In contrast, strain F208 is inactive in both Photosystems I and II reactions.

However, when the chloroplast photoreactions are examined (Table II) strain F208 has the properties expected of a mutant blocked only in Photosystem II; ferricyanide photoreduction does not occur whereas all the Photosystem I-mediated reactions take place (although the phenazine methosulphate-catalysed phosphorylation is considerably less active than that of the wild type). The only strain which behaves similarly is strain 50 which has the properties of a Photosystem I mutant in vivo but shows Photosystem I-mediated chloroplast photoreactions. However, strain 50, which has previously been shown to lack cytochrome *f* [5], is able to perform Photosystem II-mediated reactions in contrast to strain F208.

TABLE I

WHOLE CELL PHOTOREACTIONS

Rates of mutant strains are expressed as a percentage of the wild type rate. Wild type rates were: photosynthesis, 97.7 μmol CO_2 fixed/mg chlorophyll/h; glucose uptake, 9500 μmol/ml packed cell vol./h; photoreduction, 1800 μl gas uptake/ml packed cell vol./h; *p*-benzoquinone Hill reaction, 1038 μl O_2 evolved/ml packed cell vol./h.

Strain	Photo-synthesis	Light-dependent anaerobic glucose uptake	Photo-reduction	*p*-benzoquinone Hill reaction
11	0.2	34	56	0
50	0	0	0	38
F119	0.9	1	0	33
F131	1.9	23	29	0
F139	1.9	6	0	74
F199	3.1	0	0	73
F208	1.0	7	4	0

TABLE II

CHLOROPLAST PHOTOREACTIONS

Rates of mutant strains are expressed as percentage of the wild type rate. Wild type rates were: $NADP^+$ photoreduction, 25.9 and 32.6 μmol reduced/mg chlorophyll/h with water and Cl_2-indophenol/ascorbate as electron donors respectively. Methyl viologen mediated oxygen uptake, 76.5 μmol O_2 uptake/mg chlorophyll/h, phenazine methosulphate phosphorylation, 29 μmol ATP formed/mg chlorophyll/h. Ferricyanide photoreduction: 120 μmol reduced/mg chlorophyll/h.

Strain	H_2O $NADP^+$	Cl_2-indophenol ascorbate $NADP^+$	Diaminodurene ascorbate methyl viologen	Phenazine methosulphate phosphorylation	H_2O ferricyanide
11	0	35	86	40	0
50	0	78	64	48	34
F119	0	0	5	0	69
F131	0	27	95	50	0
F139	0	0	8	0	78
F199	0	0	5	0	62
F208	0	57	65	14	0

The chloroplast photoreactions of strain F208 indicated that the electron transfer chain from the point of acceptance of electrons from either reduced Cl_2-indophenol or diaminodurene to $NADP^+$ was intact, although electron transfer was blocked in Photosystem II. The loss of the capacity for in vivo cyclic phosphorylation is possibly due to the loss of cytochrome *f*. The photoreduction of methyl viologen by electrons from diaminodurene/ascorbate was found to be inhibited by 0.4 M $HgCl_2$ in strains 50, F208 and wild type. It has been shown in spinach chloroplasts that the inhibition of mercury at this concentration was due to its action on the copper in plastocyanin [23, 24]. Since strain 50 is known to be deficient in cytochrome *f* this would imply that diaminodurene/ascorbate donates electrons to *P*-700 via plastocyanin in these chloroplast fragments. As this type of mercury inhibition occurs with strain F208, this strain must be able to synthesize plastocyanin.

Soluble protein extracts were obtained from each of the mutant strains as described in Fig. 1. Prior to the chromatography of these extracts, a portion of each was examined in the analytical ultracentrifuge. With the exception of strain F208, all the extracts like that of the wild type contained a large protein which had the sedimentation characteristics of fraction 1 protein, large molecular weight and high concentration. The extract from strain F208 showed no significant contribution from such a

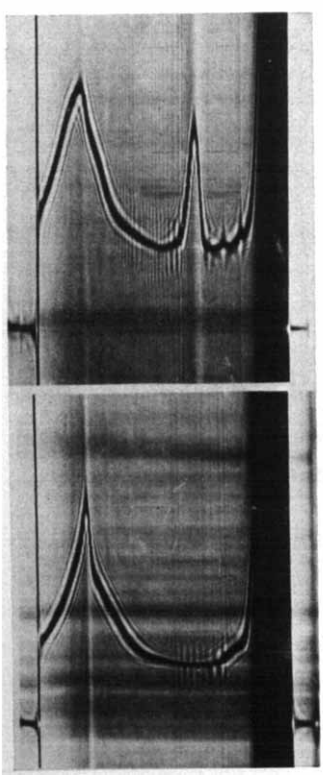

Fig. 2. Sedimentation velocity patterns of the soluble protein from wild type (upper photograph) and the F208 mutant strain (lower photograph) of *S. obliquus*. The photographs were each taken 50 min after reaching a rotor speed of 60000 rev./min. Direction of sedimentation is from left to right.

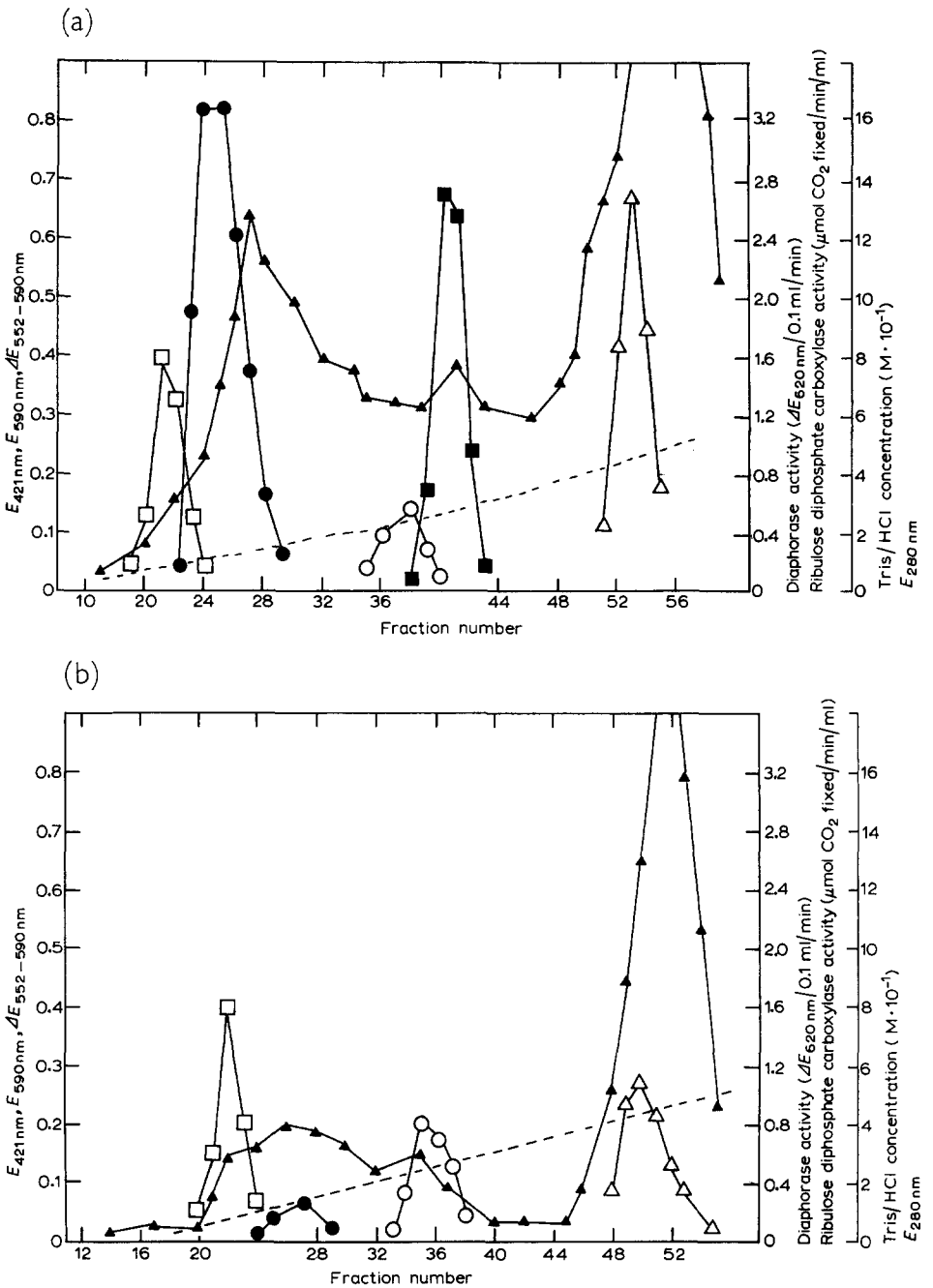

Fig. 3. Comparison of the DEAE-chromatography of protein extracts from wild type (a) and the F208 (b) strains of *S. obliquus*. □–□, NADP$^+$ ferredoxin reductase, diaphorase activity ($\Delta E_{620\,nm}$/min/ml); ●–●, ribulose diphosphate carboxylase activity (μmol CO_2 fixed/min/ml); ○–○, plastocyanin ($E_{590\,nm}$); ■–■, cytochrome *f* ($\Delta E_{552-590\,nm}$); △–△, ferredoxin ($E_{421\,nm}$); ▲–▲, $E_{280\,nm}$; – – –, Tris/HCl pH 7.5 concentration.

TABLE III

PROTEIN COMPONENTS OF MUTANT STRAINS

Plastocyanin, ferredoxin and ferredoxin-NADP$^+$ reductase were present in all strains.

Strain	*P*-700	Photosystem I protein · chlorophyll complex	Light-harvesting chlorophyll *a*/*b* protein	Soluble and bound cytochrome *f*	Fraction I protein
11	+	+	+	+	+
50	+	+	+	−	+
F119	−	−	+	−	+
F131	+	+	+	+	+
F139	−	−	+	+	+
F199	−	−	+	+	+
F208	+	+	+	−	−

protein as can be seen in Fig. 2. Since ribulose diphosphate carboxylase activity is known to be associated with fraction 1 protein [25], the chromatographic behaviour of the extracts from strain F208 and wild type on DEAE-cellulose were compared (Fig. 3). As expected, strain F208 had a very low level of ribulose diphosphate carboxylase activity, 7 % of that of the wild type, cytochrome *f* was absent; however, the other chloroplastidic proteins plastocyanin, ferredoxin and ferredoxin-NADP$^+$ reductase were present in similar concentrations to those in the wild type. Despite this low level of ribulose diphosphate carboxylase, the other enzymes unique to the Calvin cycle, phosphoribulokinase and NADP$^+$-linked glyceraldehyde-3-phosphate dehydrogenase, were present in extracts of strain F208 in concentrations of the same order as in the wild type (data not presented).

The results of a detailed examination of the proteinaceous components of all the mutant strains are shown in Table III. Extracts of all the strains which could not carry out any Photosystem I-dependent photoreactions, strains F119, F139 and F199, did not produce the protein · chlorophyll complex of Photosystem I. The lack of the reaction centre, *P*-700, of this complex was apparent from the absence of the characteristic decrease in absorption at 695 nm in the ferricyanide minus ascorbate difference spectra of the membrane fragments of these strains (Fig. 4B).

The two strains which could perform Photosystem I-mediated chloroplast reactions, but not Photosystem I-mediated whole cell reactions, strains F208 and 50, were shown by DEAE-cellulose chromatography of their extracts not to have any soluble cytochrome *f*. Moreover, the absence of membrane-bound cytochrome *f* in these strains was apparent from the decreased absorption at 552 nm of the dithionite minus ferricyanide difference spectrum of chloroplast membrane fragments when compared with those of the wild type (Fig. 4A). However, the Photosystem I protein · chlorophyll complex and *P*-700 were both present in these strains.

The classical Photosystem II mutants, strains 11 and F131, were indistinguishable from the wild type, all components were present including the Photosystem II protein · chlorophyll complex despite these strains being inactive in all the Photosystem II-mediated photoreactions. Recently this complex has been considered to be the light-harvesting chlorophyll *a*/*b* protein [26].

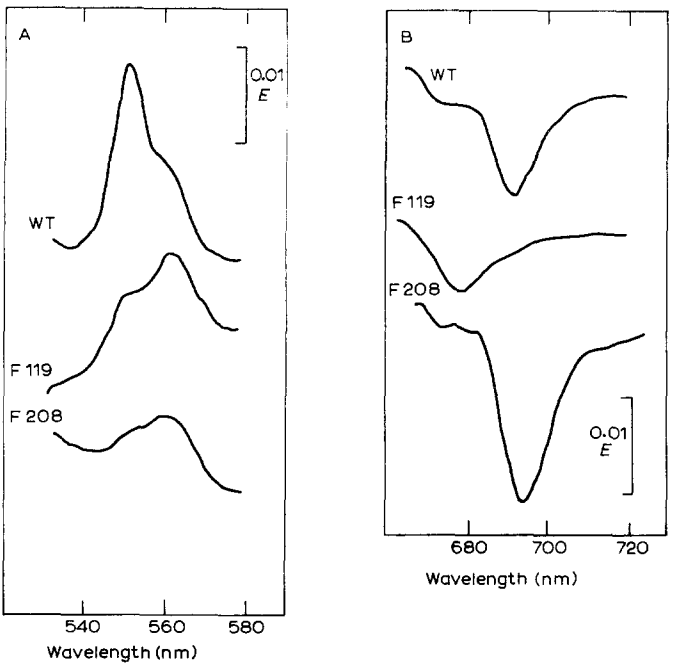

Fig. 4. A. Membrane-bound cytochromes. Dithionite vs ferricyanide difference spectra of a 2 % Triton X-100, 4 M urea, 0.05 M Tris/HCl pH 7.5 solubilized extract of crude lamellar membranes. B. *P*-700. Ferricyanide vs ascorbate difference spectra of *P*-700-ehriched membrane fragments. Upper traces, wild type; centre traces, strain F119; lower traces, strain F208.

It has been shown by Bishop and Wong [27] that the concentration of plastoquinone was much reduced from the wild type level in all their Photosystem II mutant strains. They used chlorophyll as the reference compound; however, for a number of reasons we consider that ubiquinone serves as a better reference. Ubiquinone, being a mitochondrial component, is unlikely to be affected by mutations leading to lesions in the photosynthetic apparatus. With ubiquinone as reference, plastoquinone concentrations can be compared in strains which have much reduced chlorophyll concentrations. Both quinones can be sequentially eluted from the same column prior to estimation. Molar ratios of plastoquinone: ubiquinone in some of the mutant strains are compared with the wild type in Table IV. Our results confirm those of Bishop and Wong [27], in that all strains without activity in Photosystem II reactions, strains 11, F131 and F208 have a much reduced plastoquinone content when compared with the wild type. Strain F199, a Photosystem I mutant, does have a reduced plastoquinone content but this is not of the same order as that shown by the Photosystem II mutant strains. These results support the view [27], that the loss of Photosystem II activity does not result from the inability of the algae to synthesize plastoquinone, but rather that the deficiency of plastoquinone reflects the loss of a discrete part of the Photosystem II unit within the chloroplast.

The multiple lesions of strain F208 were very similar to those reported for the ac-20 mutant strain of *C. rheinhardi* [8, 9] (Table V), these lesions are considered to arise from the inability of this strain to bring about the normal biogenesis of chloro-

TABLE IV

PLASTOQUINONE LEVELS OF STRAINS OF *SCENEDESMUS OBLIQUUS*

Strain	Growth conditions	Molar ratio plastoquinone/ ubiquinone	Percentage of heterotrophic wild type
WT	Heterotrophic	2.00±0.18	100
WT	Mixotrophic	2.69±0.14	135
11	Heterotrophic	0.78	39
F131	Heterotrophic	0.48	24
F199	Heterotrophic	1.48	74
F208	Heterotrophic	0.46	23

TABLE V

COMPARISON OF F208 AND ac-20

Values expressed as percentage of the wild type rates: +, present; −, absent.

	ac-20	F208
Photosynthetic rate	3 %	1 %
H_2O/$NADP^+$	2 %	−
Cl_2-indophenol/$NADP^+$	60 %	57 %
Phenazine methosulphate-dependent cyclic phosphorylation	68 %	14 %
H_2O/ferricyanide	21 %	−
P-700	+	+
Ferredoxin	+	+
Ferredoxin $NADP^+$ reductase	+	+
Cytochrome *f*	low	+
Ribulose diphosphate carboxylase	2 %	7 %
Plastoquinone	+	23 %
Plastocyanin	+	+

plast ribosomes. In view of this it was decided to fractionate the ribosomal RNA of strain F208. Details of the electrophoresis of the RNA isolated from strain F208 and wild type after staining with methylene blue is shown in Fig. 5. The wild type gels showed 4 slowly migrating bands corresponding to the 25 S and 18 S RNA species derived from 80 S cytoplasmic ribosomes, and 23 S and 16 S RNA species derived from 70 S chloroplast ribosomes. The concentration of each species was estimated from the area under each of the peaks, the 23 S and 16 S species in extracts of strain F208 were consistently found to be reduced in concentration to approx. 40 % of the wild type level assuming that the level of the 25 S and 18 S species were unaffected. This does not suggest as great a deficiency of chloroplast ribosomes in strain F208 as that reported for ac-20 [10], which when grown mixotrophically has only 8 % of the chloroplast ribosomes found in the wild type. However, Boynton et al. [28] found that their stock of strain ac-20 was in fact a double mutant, carrying both the original ac-20 mutation and a new spontaneous mutation, cr-1. When looked at separately, both of these mutations resulted in greatly reduced amounts of intact chloroplast

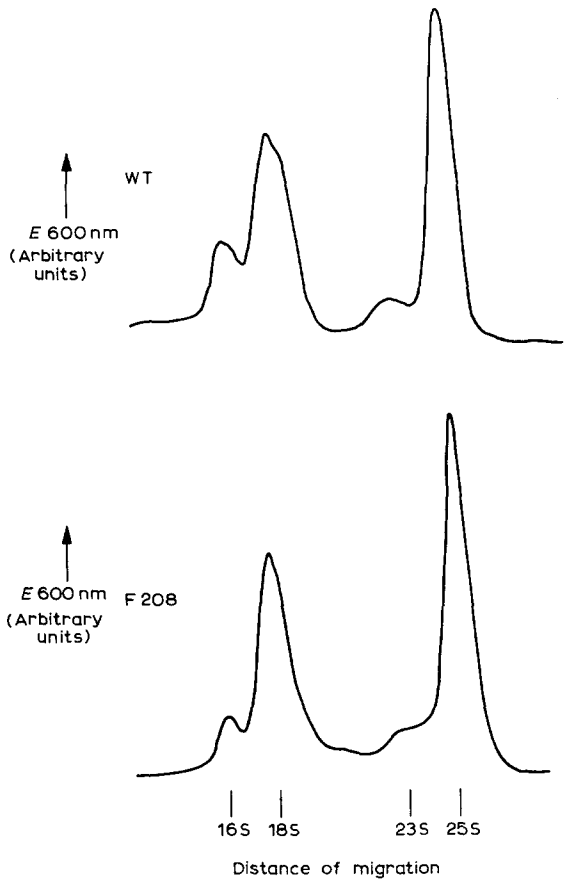

Fig. 5. Polyacrylamide electrophoresis of ribosomal RNA species extracted from wild type (upper trace) and strain F208 (lower trace). Direction of migration was from right to left; RNA species were localized by staining the gels with methylene blue.

ribosomes. Unlike ac-20, the cr-1 strain is able to synthesise the large subunit of the chloroplast ribosome and RNA extracts of this strain do not contain any 16 S RNA although 23 S RNA is present. This group [29, 30] have also shown that both these mutant strains have only very low levels of ribulose diphosphate carboxylase when grown mixotrophically. However, they also found that in mixotrophic ac-20 chloroplast ribosomes were present to the extent of 30 % of the wild type level, a three-fold increase in the value reported by Goodenough and Levine [10].

The abnormal chloroplast morphology of strain ac-20 had been described by both groups. In mixotrophically grown cells Goodenough and Levine [10] report haphazard arrays of unfused discs as well as tightly aggregated vesicles, however Bourque et al. [29] describe an extensive highly-ordered lamellar system consisting of abnormally large diameter stacks, resembling giant grana. These giant grana were only found by Goodenough and Levine [10] when mixotrophic cells were transferred to minimal medium and incubated in the dark for a few hours. In the case of our mutant

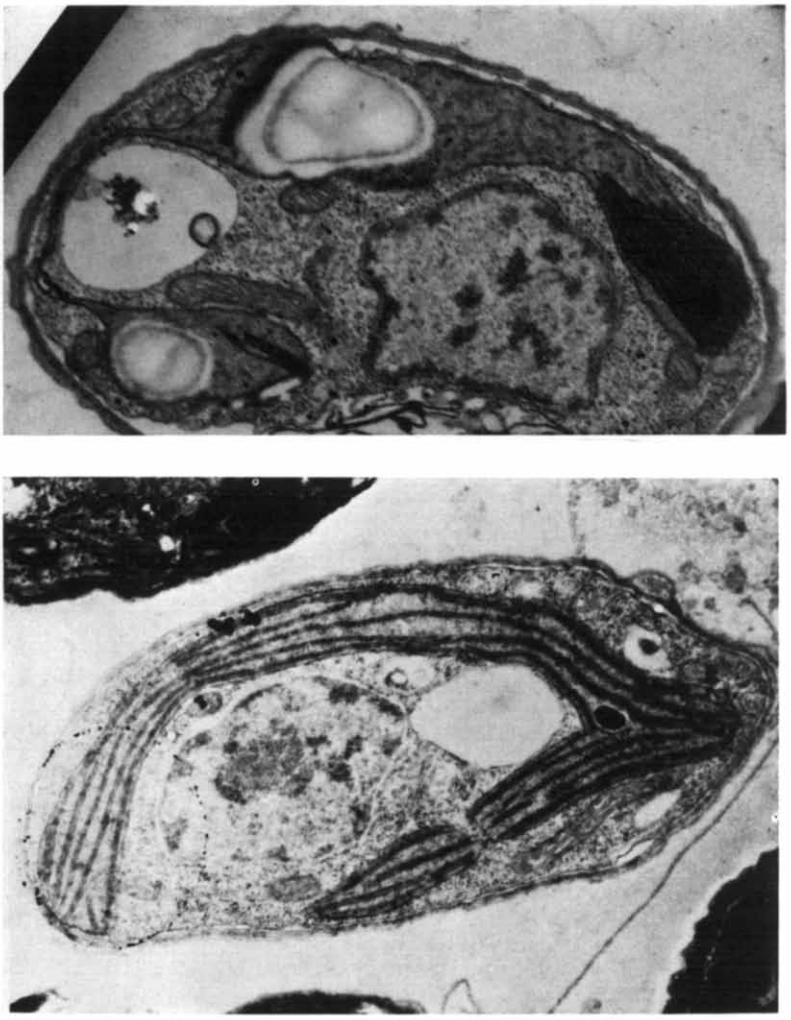

Fig. 6. Ultrastructure of heterotrophically grown strains of *S. obliquus*; strain F208 (upper photograph), wild type (lower photograph).

strains, all had a chloroplast structure indistinguishable from that of the wild type apart from strain F208. In this strain a large number of thylakoids were found to be stacked together when grown under heterotrophic conditions (Fig. 6).

Interference with chloroplast protein synthesis in *C. rheinhardi* either by mutation or the use of antibiotics gives rise to well-defined symptoms which resemble those shown by F208 strain of *S. obliquus*. Addition of spectinomycin and chloramphenicol, inhibitors of chloroplast ribosome function, to synchronous cultures of *C. rheinhardi* prevented the formation of cytochrome *f* (*c*-553), Photosystem II activity and ribulose diphosphate carboxylase activity [31]. Furthermore, normal chloroplast membrane organization and Photosystem II activity were not produced when the y-1

mutant of *C. rheinhardi* was transferred into the light in the presence of chloramphenicol [32]. Hence the phenotype of strain F208 is as would be expected to result from a deficiency of protein synthesis by the chloroplast.

The photochemical activities of strain F208, in conjunction with those reported for ac-20 [8–10] would imply that this lesion causes a drastic fall in Photosystem II activity, much more marked than that seen in strain 50 which is only lacking in cytochrome *f* and has a third of the wild type activity in Photosystem II. Moreover, in strain F208 the extent of Photosystem I reactions are also diminished to a greater extent than in strain 50, although not to the same degree as the Photosystem II reactions.

However, caution must be exercised in relating the photosynthetic properties of strain F208 to a lack of chloroplast ribosome activity since it is possible that the phenotype of this strain could result from a multiple mutation. Unfortunately this possibility cannot be tested, since no sexual phase in the life cycle of *S. obliquus*, which can be controlled under laboratory conditions, is known at present; consequently any genetic analysis is prevented.

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