CHLOROPLAST STRUCTURE AND FUNCTION IN *ac-20*, A MUTANT STRAIN OF *CHLAMYDOMONAS REINHARDI*

II. Photosynthetic Electron Transport

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ABSTRACT

Photosynthetic electron transport is markedly affected in mixotrophic cells of *ac-20* because they lack the capacity to form the wild-type level of cytochrome 559, as well as Q, the quencher of fluorescence of photochemical system II. The other components of the electron-transport chain, as well as reactions dependent upon photochemical system I, are unaffected in the mutant strain. These observations are discussed in terms of the previously reported effects of the *ac-20* mutation on CO_2 fixation and ribulose-1,5-diphosphate carboxylase activity.

INTRODUCTION

In the preceding paper (19) it was shown that *ac-20*, a mutant strain of *Chlamydomonas reinhardi*, lacks the wild-type capacity to fix carbon dioxide by photosynthesis, that it lacks the wild-type level of RuDP[1] carboxylase, an enzyme essential for photosynthetic carbon dioxide fixation, and that compared with phototrophically-grown cells, mixotrophically-grown cells have a lower rate of carbon dioxide fixation as well as a lower level of the carboxylation enzyme. In this paper we describe studies of the mutant strain's photosynthetic electron-transport chain. It is shown that in mixotrophic *ac-20*, the rate of electron flow from water through PS II and PS I to NADP is slower than in wild type. Cytochrome 559, a component known to be associated with PS II (15), and Q, the quencher of fluorescence of PS II, are both diminished in amount or activity in the mutant strain, whereas the other known components of the transport chain exhibit normal activities or are present in normal amounts. The extent of photosynthetic electron transport and the levels of cytochrome 559 and Q are both affected by mixotrophic versus phototrophic growth conditions, in a manner similar to that described for CO_2 fixation and RuDP carboxylase.

MATERIALS AND METHODS

Organisms and Culture Conditions

The wild-type strain (137c) and the mutant strain, *ac-20*, of *C. reinhardi* were used in the experiments described here. They were cultured under the conditions described in the previous paper (19). Transfer experiments were carried out as described in that paper.

[1] Abbreviations used: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; DPIP, 2,6-dichlorophenol-indophenol; PMS, phenazine methosulfate; PS I and PS II, photochemical systems I and II, respectively; RuDP, ribulose-1,5-diphosphate.

Preparation of Chloroplast Fragments

Chloroplast fragments for the study of photosynthetic electron transport were prepared by the ultrasonic disruption of cells (15) and for the study of cyclic and noncyclic photosynthetic phosphorylation by disrupting the cells by grinding them in sand (9).

Chlorophyll Content and Cell Number

The chlorophyll content of chloroplast fragments and of whole cells was determined by a modification (1) of the method of Mackinney (16). Cell numbers were obtained with the aid of a hemacytometer.

Reactions of the Photosynthetic Electron-Transport Chain

The Hill reaction with either DPIP or NADP as the electron acceptor and the photoreduction of NADP with the DPIP-ascorbate couple were measured in chloroplast fragments as described elsewhere (6, 9).

Photosynthetic Phosphorylation

Cyclic photosynthetic phosphorylation with PMS as the electron carrier, and noncyclic photosynthetic phosphorylation with ferricyanide as the electron acceptor, were measured in chloroplast fragments as previously described (7, 9).

Fluorescence

The fluorescence of PS II was measured in the manner described by Yamashita and Butler (20).

Components of the Photosynthetic Electron-Transport Chain

Total carotenoids were determined as described by Krinsky and Levine (12). Ferredoxin was assayed according to methods previously described (18), using the crude supernates from a 140,000 *g* centrifugation of sonically disrupted cells. A known number of cells having a known amount of chlorophyll per cell was used. Chloroplast fragments prepared from wild-type cells were used in the assay and purified ferredoxin-NADP reductase prepared from wild-type cells was present in excess. The amount of ferredoxin (units) in the crude extracts was expressed in terms of the quantity of protein necessary to photoreduce 1 μmole of NADP.

Ferredoxin-NADP reductase in the crude preparations was assayed according to the procedure of Avron and Jagendorf (2), where one unit is the amount of protein in the crude extract that catalyzes the reduction of 1 μmole of DPIP.

Plastocyanin content was not determined directly, but its presence was ascertained by measuring the photoreduction of NADP with DPIP-ascorbate

TABLE I

Reactions of the Photosynthetic Electron-Transport Chain of ac-20 and Wild-Type C. reinhardi

Strain	Growth condition	NADP-Hill reaction		DPIP-Hill reaction		NADP photoreduction with DPIP-ascorbate	
		µmoles NADP reduced/		*µmoles DPIP reduced/*		*µmoles NADP reduced/*	
		10^8 cells per hr	*mg chlorophyll per hr*	*10^8 cells per hr*	*mg chlorophyll per hr*	*10^8 cells per hr*	*mg chlorophyll per hr*
Wild type	Phototrophic	60.0	250	50.5	228	23.0	97.0
	Mixotrophic	61.0	149	61.0	174	18.0	51.0
ac-20	Phototrophic	6.8	57	21.5	180	10.0	81.0
	Mixotrophic	1.2	5	11.0	45	8.0	31.0

For the Hill reaction with DPIP, the cuvette in the sample compartment of the spectrophotometer contained chloroplast fragments (10 μg chlorophyll) prepared by the sonication of cells, and the following in μmoles: potassium phosphate, pH 7.0, 20; KCl, 40; $MgCl_2$, 5.0; and DPIP, 0.1. The final volume was 2.0 ml. The DPIP was omitted from the control cuvette in the reference compartment.

For the Hill reaction with NADP, the cuvette contained chloroplast fragments (10–15 μg chlorophyll) prepared by the sonication of cells, and the following in μmoles: potassium phosphate, pH 7.0, 20; KCl, 40; $MgCl_2$, 5; NADP, 0.5; and ferredoxin prepared from wild-type *C. reinhardi*, 0.005. Half a unit of ferredoxin-NADP reductase, prepared from wild-type *C. reinhardi*, was also added. The final volume was 2.0 ml. Ferredoxin, ferredoxin-NADP reductase, and NADP were omitted from the control cuvette.

For the photoreduction of NADP from the DPIP-ascorbate couple, the reaction mixture contained, in addition to the components for the NADP Hill reaction, the following in μmoles: DPIP, 0.1; sodium ascorbate, pH 7.0, 10; and DCMU, 0.2. The control cuvette contained everything but ferredoxin, ferredoxin-NADP reductase, and NADP.

The reactions were run at 25°C.

couple. This reaction in *C. reinhardi* is known to require the presence of plastocyanin (9).

Total quinones including plastoquinones and ubiquinone were determined from heptane extracts of cells (3).

The content of cytochromes 553, 559, and 564 was determined spectrophotometrically with an Aminco-Chance double beam spectrophotometer (American Instrument Co., Inc., Silver Spring, Md.). Both light-induced and chemically-induced absorbance changes at their respective α bands were measured (10, 15). An extinction coefficient, reduced-minus-oxidized, of 20 cm^2/μmole cytochrome was assumed (18). P-700 was determined spectrophotometrically (7).

RESULTS

Reactions of the Photosynthetic Electron-Transport Chain

The Hill reaction, in which several different oxidants (or electron acceptors) can be photoreduced by either whole cells or chloroplast fragments using water as the source of electrons, is a measure of the activity of the photosynthetic electron-transport chain. The terminal acceptor for the chain is NADP, and its photoreduction is a measure of the over-all operation of the chain; accordingly, its photoreduction requires the operation of both PS I and PS II (4, 11, 14). The data in Table I show that phototrophic and mixotrophic *ac-20* are unable to photoreduce NADP at a rate comparable to that of the wild-type strain. This difference is more apparent on a cell basis than on a chlorophyll basis, because the strains vary considerably in chlorophyll content (19). Furthermore, the Hill reaction with other oxidants (i.e., DPIP) is also low in *ac-20* (Table I). Just as with CO_2 fixation and RuDP carboxylase activity, the mixotrophic *ac-20* cells exhibit much more drastic defeciencies than the phototrophic *ac-20* cells.

TABLE II

Photosynthetic Phosphorylation by Mixotrophic Cells of ac-20 and Wild-Type C. reinhardi

Strain	Ferricyanide reduced	ATP formed	P/2e⁻	PMS cyclic phosphorylation
	μmoles/hr per mg chlorophyll	*μmoles ATP formed/hr per mg chlorophyll*		*μmoles ATP formed/hr per mg chlorophyll*
Wild type	876	105	0.24	186
ac-20	190	51	0.54	274

The reactions were run at 25°C in 25-ml Erlenmeyer flasks. The reaction mixture (2 ml) contained chloroplast fragments (40–80 μg chlorophyll) prepared from cells disrupted by grinding with sand, and the following in μmoles: glycylglycine buffer, pH 8.0, 40; $MgCl_2$, 10; ADP, pH 7.5, 5; AMP, pH 7.5, 5; and potassium phosphate buffer, pH 8.0, 10, containing 0.5–1.0 μCi $^{32}P_i$. For cyclic photosynthetic phosphorylation, the mixtures contained 0.067 μmoles of phenazine methosulfate and 0.02 μmoles of DCMU. For noncyclic photosynthetic phosphorylation, 2 μmoles of potassium ferricyanide were added. All reaction flasks were flushed continuously with nitrogen throughout the experiment. Reactions were terminated after 3 min of illumination (30,000 lux) by turning off the lights and by adding 0.2 ml of 20% trichloroacetic acid to each flask. Dark controls were run in flasks covered with black tape.

TABLE III

Components of the Photosynthetic Electron-Transport Chain of Mixotrophically-Grown ac-20 and Wild-Type C. reinhardi

Component	*ac-20*	Wild type
Chlorophyll *a* ($\mu g/10^6$ cells)	1.20	2.88
Chlorophyll *b* ($\mu g/10^6$ cells)	0.80	1.23
P-700 (mole P700/moles chlorophyll)	1:2580	1:2490
Carotenoid ($\mu g/10^6$ cells)	0.29	0.62
Ferredoxin (units)	0.83	1.24
Ferredoxin-NADP reductase (units)	5.06	3.58
Plastocyanin	+	+
Plastoquinone (mole plastoquinone/moles chlorophyll)	1:64	1:46
Cytochromes (mole cytochrome/moles chlorophyll)		
553	1:2000	1:1440
559	0	1:840
564	1:366	1:290

The photoreduction of NADP can be achieved without water as the source of electrons in a reaction that requires only PS I, if electrons are supplied from an external source such as the DPIP-ascorbate couple. This reaction in mixotrophic *ac-20* does not differ markedly from that of phototrophic *ac-20*, nor from that of the wild type strain (Table I). From a knowledge of the photosynthetic electron-transport chain of *C. reinhardi*

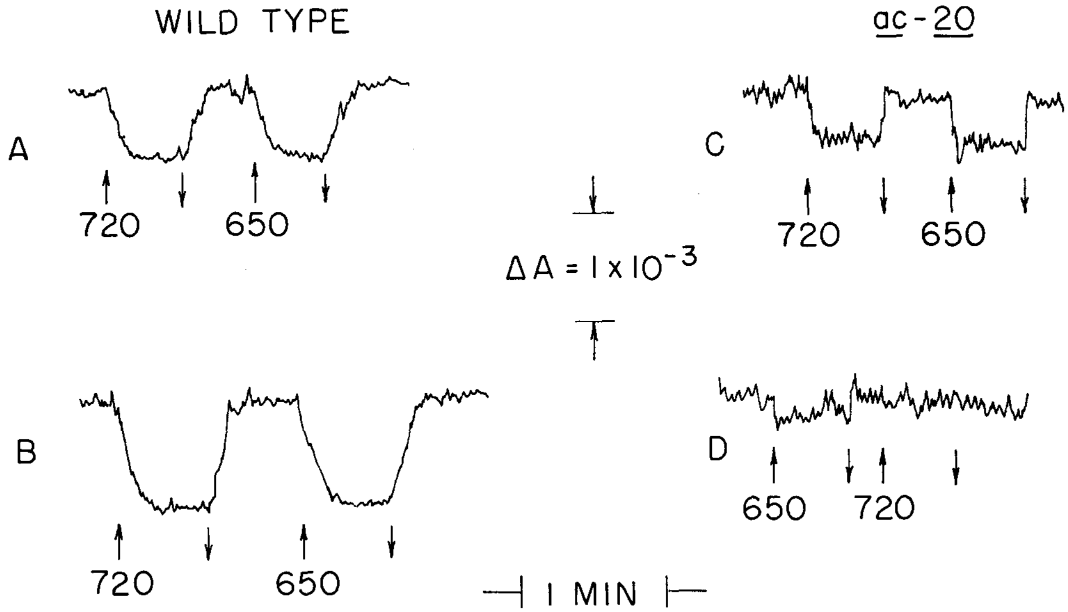

FIGURE 1 Light-induced absorbance changes in chloroplast fragments of mixotrophically-grown wild type and *ac-20*. The preparations contained 1 μmole of ascorbate to reduce cytochromes 553 and 559. In *A* and *C*, the measuring wavelength was 553 nm, and in *B* and *D* it was 559 nm. The reference wavelength was 542 nm. The wavelengths of actinic light were 650 and 720 nm. Arrows pointing upward indicate actinic light ON; arrows pointing downward indicate actinic light OFF. Chloroplast fragments equivalent to a chlorophyll concentration of 100 μg/ml were present in a volume of 1.6 ml. The temperature was 25°C. Parts *A* and *B* show for wild type the light-induced oxidation of cytochromes 553 and 559, respectively. Part *C* shows the light-induced oxidation of cytochrome 553 in chloroplast fragments of *ac-20*. Part *D* shows that in *ac-20* there is no light-induced oxidation of cytochrome 559.

(10, 14), this result indicates that the photosynthetic electron-transport chain is intact between plastocyanin and NADP, and that the reduced activity of some component or components lying between water and plastocyanin limits the NADP Hill reaction in the mutant strain.

Photosynthetic Phosphorylation

Measurements of cyclic and noncyclic photosynthetic phosphorylation agree with the findings described in the preceding paragraphs. Cyclic photosynthetic phosphorylation, dependent upon PS I and not PS II, is normal in the mutant strain, whereas noncyclic photosynthetic phosphorylation, which depends on both photochemical systems, is markedly reduced (Table II).

Components of the Photosynthetic Electron-Transport Chain

The results of an analysis comparing the components of the photosynthetic electron-transport chain of mixotrophic wild type and mixotrophic *ac 20* are given in Table III. Also shown are the chlorophyll and carotenoid content of the mutant strain. It is seen in Table III and in Figs. 1 and 2 that cytochrome 559 is not detected in *ac-20*, either by light- or chemically-induced absorbance changes whereas, with the exception to be noted below, known components are present in amounts essentially equivalent to those found for the wild-type strain.

Fluorescence Properties

The levels of fluorescence in cells of the wild-type and the mutant strain are compared in Fig. 3. It can be seen that the initial level of fluorescence (i.e., the fluorescence detected by the weak measuring beam of 69 ergs/cm^2 per sec) is higher in mixotrophically-grown cells of the mutant strain than in mixotrophically-grown cells of the wild-type strain. The fluorescence yield in the mutant cells is not, however, appreciably increased upon

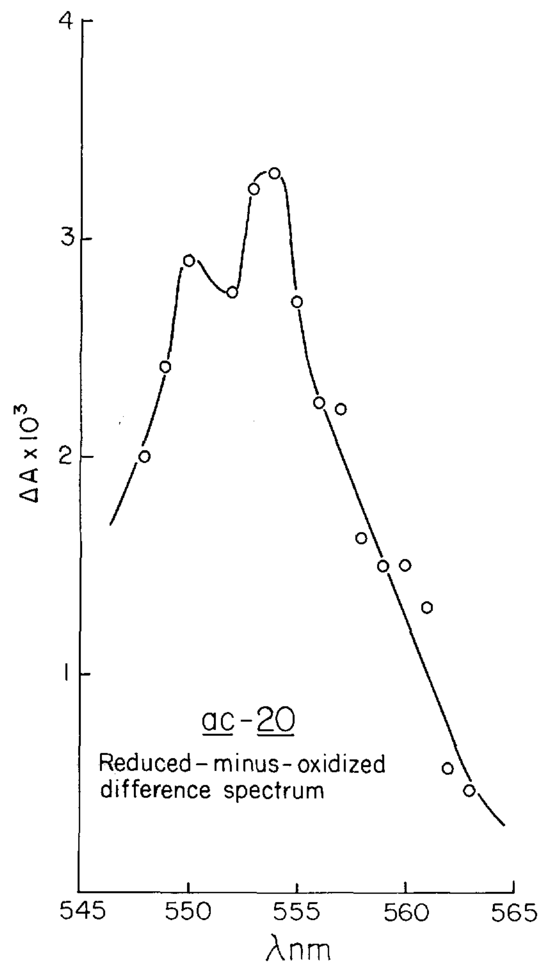

FIGURE 2 Reduced-minus-oxidized difference spectrum for chloroplast fragments of mixotrophically-grown *ac-20*. The reducing agent was ascorbate, and the oxidizing agent was potassium ferricyanide. The difference in absorbance of a reduced and of an oxidized sample was determined at the wavelengths indicated on the abscissa. The reference wavelength was 542 nm. Chlorophyll concentration in a 1.6 ml suspension of chloroplast fragments was 100 μg/ml, and the temperature was maintained at 25°C. Note that the peaks representing the α-bands of cytochromes 550 (presumably the mitochondrial cytochrome *c*) and 553 (the chloroplast *c*-type cytochrome) are present and prominent, whereas there is no peak at 559 nm, the position of the α-band of the chloroplast cytochrome 559.

illumination with the relatively intense actinic light (4.2×10^4 ergs/cm² per sec), in contrast to wild type. It can also be seen that the addition of DCMU does not have an appreciable effect on the over-all fluorescence yield of mixotrophic *ac-20*. Results of this sort have been interpreted (5, 13, 17) to mean that Q, the quencher of fluorescence of PS II, is either missing or inactive. It is of interest that other mutant strains of *C. reinhardi* that lack cytochrome 559 also appear to lack Q (13).

Phototrophically-grown cells of *ac-20* have fluorescence properties that more closely resemble those of mixotrophically- or phototrophically-grown cells of the wild-type strain (Fig. 3); the yield is increased upon actinic illumination, although it dces not reach the wild-type level, and it can be further increased upon the addition of DCMU. Thus the fluorescence data also indicate that photosynthetic electron transport is not greatly impaired in phototrophically-grown cells of *ac-20*.

Transfer Experiments

The change in the level of RuDP carboxylase that occurs when mixotrophic cells of *ac-20* are transferred to phototrophic growth conditions (19) is paralleled by changes in photosynthetic electron transport. Fig. 4 gives the results of light-to-light and light-to-dark transfer experiments in which the course of NADP photoreduction was followed. Similar results were obtained in experiments that followed the course of photoreduction of DPIP. It will be noted that in comparison with the recovery of RuDP carboxylase activity and CO_2 fixation (19), the recovery of electron transport in a light-to-light transfer occurs after a shorter lag, and the rate of recovery appears to be more rapid.

The recovery of photosynthetic electron transport in transfer experiments is inhibited by antibiotics that block protein synthesis (experiments to be reported elsewhere). Thus, like the recovery of RuDP carboxylase activity, the recovery of photosynthetic electron transport appears to require protein synthesis and is not a simple activation process.

DISCUSSION

The rate of photosynthetic CO_2 fixation is reduced in *ac-20* because of a deficiency of RuDP carboxylase (19). The data presented in this paper reveal that the rate of photosynthetic electron transport is also reduced and that this is because of a deficiency of cytochrome 559 and, most likely, of Q. The limiting step in photosynthesis by mixotrophic cells of *ac-20*, however, is probably in the fixation of CO_2, since RuDP carboxylase activity

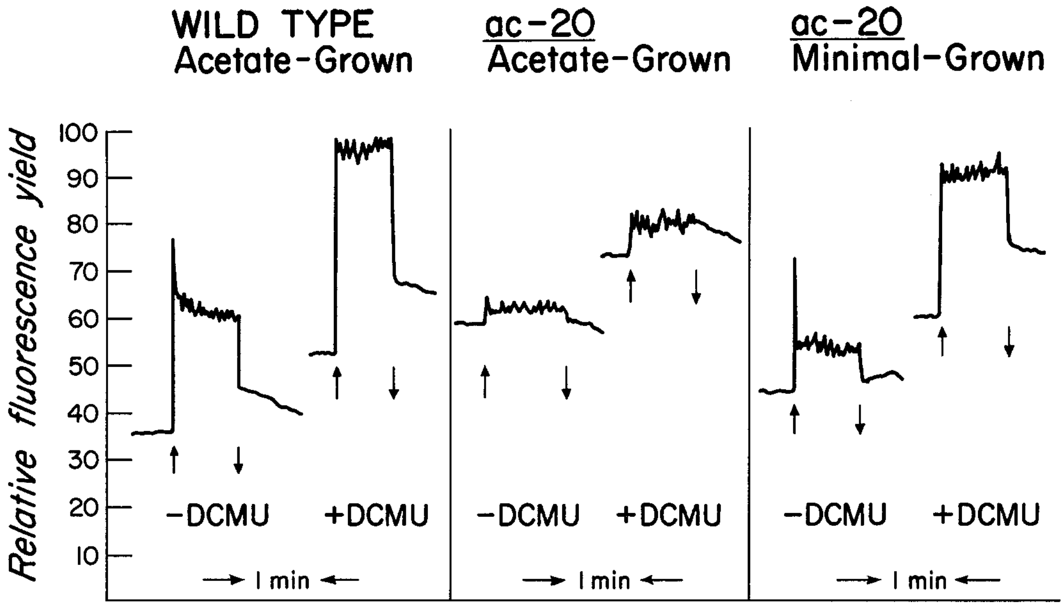

FIGURE 3 Fluorescence yield of chloroplast fragments of mixotrophically- and phototrophically-grown *ac-20* and wild type. The fluorescence was induced by 650 nm actinic light (4.2×10^4 ergs/cm^2 per sec). Arrow pointing upward indicates actinic light ON; arrow pointing downward indicates actinic light OFF. The chloroplast fragments were contained in 1 ml, and the chlorophyll concentration was 48 μg/ml. The temperature was 23°C.

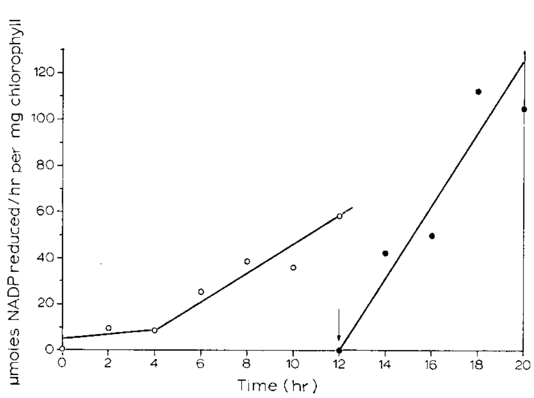

FIGURE 4 The recovery of the NADP Hill reaction in a transfer experiment. At time 0, mixotrophically-grown cells were transferred to minimal medium and placed in the light (light-to-light transfer) or the dark (light-to-dark transfer). The results of the light-to-light transfer (open circles) show that a lag of 4 hr precedes the onset of Hill reaction recovery. The results of the light-to-dark transfer (filled circles) show no Hill reaction recovery after 12 hr of incubation in the dark. However, activity recovers immediately when the cells are placed in the light (arrow).

is more drastically affected than is the rate of photosynthetic electron transport (compare Table II in the previous paper with Table I in this paper). The levels of other, known, components of the alga's photosynthetic electron-transport chain do not appear to differ appreciably from wild-type levels.

The effect of acetate on the rate of photosynthetic electron transport and on the level of cytochrome 559 is analogous to its effect on the rate of CO_2 fixation and RuDP carboxylase activity, in that the transfer of mixotrophic cells to minimal medium results in an increase in the rate of transport and in the level of cytochrome 559.

Clearly, cells of *ac-20* possess at least three anomalies that affect their photosynthetic capacity; as will be shown (8), the organization of chloroplast membranes is also affected, and there is also a deficiency in chloroplast ribosomes.

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