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CHLOROPLAST REACTIONS OF PHOTOSYNTHETIC MUTANTS OF *SCENEDESMUS OBLIQUUS*

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SUMMARY

Partial reactions of photosynthesis were examined in the wild-type strain and six photosynthetic mutants of *Scenedesmus obliquus*, Strain D_3. A reproducible technique for the isolation of active chloroplast fragments from Scenedesmus was developed. Several chloroplast reactions and two reactions *in vivo* (anaerobic glucose assimilation and non-photochemical nitrite reduction) were examined. Chloroplast preparations of Mutant 8 would neither reduce $NADP^+$ nor perform cyclic photophosphorylation although Hill reaction activity with low redox potential oxidants, such as 2,6-dichlorophenolindophenol and ferricyanide, was present. These findings confirm earlier results which demonstrated that Mutant 8 is a System I mutant.

Mutants 11, 40, and a′ were found to be defective in System II since they lacked Hill reaction activity but possessed normal cyclic photophosphorylation, 2,6-dichlorophenolindophenol–ascorbate-mediated reduction of $NADP^+$, and photoreduction activities *in vivo*. Chloroplasts of Mutants 26 and 50 performed the same chloroplast reactions as the System II mutants, but are not typical System II mutants since they lacked photoreduction activity *in vivo*, a process requiring only System I. Although these mutants performed cyclic photophosphorylation *in vitro*, no evidence of a photophosphorylation *in vivo* was obtained. Provisionally, the defects in Mutants 26 and 50 were located in the electron transport chain between the two light reactions.

INTRODUCTION

The application of selected mutant organisms to studies of biochemical pathways is a common practice. However, only recently has this experimental approach been applied to studies on the general mechanisms of photosynthesis. The realization of the many partial reactions peculiar to photosynthesis, such as photophosphorylation, photoreduction, and the Hill reaction allows for an ideal situation where mutant studies might profitably be employed. The production and isolation of photosynthetic mutants, by BISHOP[1,2] in Scenedesmus, LEVINE[3] in Chlamydomonas, and most recently by RUSSELL AND LYMAN[4] in Euglena provide a means for examining

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

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many of these biochemical events leading to the photosynthetic incorporation of carbon dioxide into cellular mass. Such mutants may be used to verify existing hypotheses as well as to suggest new ones. (Photosynthetic mutants are defined by their inability to utilize light in the fixation of CO_2 and are to be differentiated from the more commonly occurring pigment mutants.)

BISHOP[2] has previously described properties of several photosynthetic mutants of *Scenedesmus obliquus*, Strain D_3, and has classified them with respect to their ability to utilize *p*-benzoquinone in the Hill reaction and hydrogen in photoreduction. (Photoreduction, the cellular process utilizing hydrogen for the light-dependent reduction of CO_2, is to be distinguished from the chloroplast photoreductions discussed below.)

Mutants 11, 40, and a', so-called oxygen mutants, lack photosynthetic and quinone–Hill reaction activity but have a nearly normal photoreduction activity. Mutant 8, which lacks both photosynthesis and photoreduction, but possesses the Hill reaction, has been isolated and its characteristics studied in some detail. Two additional mutants (26, 50), lack all three reactions mentioned above; Mutant 50 is described here for the first time.

Photochemically active chloroplast preparations, consisting of both whole and fragmented chloroplasts, have been made from the wild type and the six mutant phenotypes (8, 11, 26, 40, 50 and a') of *Scenedesmus obliquus*, Strain D_3. The capacity of these chloroplast preparations to photoreduce several oxidants and to generate ATP in response to light has been examined. Nitrite reduction and anaerobic glucose assimilation were also measured in some of these mutants in an attempt to define the role of the photosynthetic light reactions in photoreduction.

METHODS

Algal culture

The algae were cultured heterotrophically on nitrate medium supplemented with 0.5 % glucose and 0.25 % yeast extract[5] and were maintained at 25° in erlenmeyer flasks kept in darkness on a rotating shaker. 5-day-old cultures were used since they were found to be at an optimal stage both for *in vivo* measurements and for the preparation of chloroplasts. All cultures were examined on a Gilson Oxygraph before use to detect any possible revertants of the mutants. Only those mutant cultures which showed no O_2 evolution upon illumination were used in the experiments described below.

The packed cell volume of the algal cultures was determined by centrifugation of aliquots of the culture medium. The collected cells were then transferred to a cytocrit tube with 0.06 M HCl (to dissolve inorganic precipitates) and centrifuged for 5 min at 1000 × *g*. Cell concentration in the 5-day-old cultures was approx. 10 μl packed cell volume per ml.

Chloroplast preparation

The algal cells were harvested by centrifugation and were washed twice in a buffer solution (0.02 M potassium phosphate buffer, 10^{-5} M EDTA (pH 7.0) for chloroplast photoreductions; 0.4 M sucrose, 0.01 M NaCl, 0.05 M Tris–HCl, 10^{-3} M mercaptoethanol, $5 \cdot 10^{-5}$ M EDTA (pH 7.8), for photophosphorylation). 20 ml of cell suspension containing approx. 5 ml packed cell volume were placed in a 50-ml

stainless-steel cup which was then filled to the top with 0.35-mm diameter glass beads. The cells and glass beads were vibrated at full power on a Vibrogen–Zellmühle for 5 min while ice–water was circulated around the stainless-steel cup. The contents of the cup were then filtered through a coarse, fritted-glass filter to remove the glass beads which were subsequently washed with additional buffer for complete recovery of the broken cells. The chloroplast fraction was obtained by centrifugation of the filtrate at 1000 × *g* for 10 min and of this supernatant at 15000 × *g* for 15 min at 0°.

The 15000 × *g* pellet was suspended with a glass homogenizer in another buffer solution (0.4 M sucrose, 0.01 M NaCl, 0.05 M Tris–HCl (pH 7.8), for chloroplast photoreductions; 0.4 M sucrose, 0.01 M NaCl, 0.05 M Tris–HCl, $5 \cdot 10^{-5}$ M EDTA (pH 7.8), for photophosphorylation). Enough suspending buffer solution was added to give a chlorophyll concentration of approx. 200 μg/ml for chloroplast photoreductions and 400–600 μg/ml for photophosphorylation. The chloroplasts were examined for photochemical activity either immediately or following storage at −30°.

Spinach chloroplasts for photophosphorylation experiments were isolated by the procedure described above for Scenedesmus with the exception that the spinach cells were broken by grinding the leaves in a mortar and pestle with 0.35-mm diameter glass beads.

Chlorophyll was measured using the absorption coefficients given by G. MACKINNEY (see ref. 6) for chlorophylls *a* and *b* in methanol. Spinach photosynthetic pyridine nucleotide reductase was prepared with the method of SAN PIETRO AND LANG[7].

Chloroplast photoreductions

The reaction mixtures for the various chloroplast photoreductions are given in Tables I and II. The reactions were assayed at room temperature with a Zeiss PMQ II spectrophotometer. A 1000-W tungsten filament lamp, filtered with a red Corning glass cut-off filter (2-61) and a 5-cm water bath, was used as the actinic light source. The light beam was focused on the top of a 1 cm × 1 cm × 4 cm cuvette. The light intensity was measured with a small surface bolometer (Model 65-Radiometer, Yellow Springs Instrument Co.) and was approx. 10^6 ergs·sec^{-1}·cm^{-2}. The reactions were followed by recording the absorbance before, during, and after actinic illumination. The net light-dependent rate of absorbance change was used to determine the corresponding chloroplast photoreduction rate.

Coefficients used to convert absorbance changes (ΔA) in a 1-cm cuvette into μmoles substrate reduced are:

μmoles cytochrome *c*/ml = 0.0510 $\Delta A_{550\,nm}$ (ref. 8); μmoles NADPH/ml = 0.2029 $\Delta A_{340\,nm}$ (ref. 9); μmoles DCIP/ml = 0.0563 $\Delta A_{610\,nm}$; μmoles ferrocyanide/ml = 0.962 $\Delta A_{410\,nm}$*.

The subscripts indicate the wavelengths at which the reactions were followed. The coefficient used for 2,6-dichlorophenolindophenol (DCIP) reduction was determined with a fresh solution of high purity DCIP.

Photophosphorylation

Chloroplasts for photophosphorylation assays were always used immediately after preparation. Assays were carried out in 50-ml erlenmeyer flasks on a Gilson

* J. COWLES, personal communication.

respirometer equipped with an adapter bar to hold the flasks. The standard assay mixture contained (in 3 ml) 45 μmoles Tris–HCl (pH 7.8), 12 μmoles $MgCl_2$, 60 μmoles NaCl, 12 μmoles potassium phosphate buffer (pH 7.8), 1.2 μmole sucrose, 50 μmoles ascorbate, 12 μmoles ADP, 0.09 μmole phenazine methosulphate, 10–50 μC ^{32}P, and 40 μg chlorophyll in 0.1 ml suspending medium. The actinic light was filtered through a sheet of red plastic passing wavelengths greater than 600 nm and providing an intensity of approx. 10^5 ergs·sec^{-1}·cm^{-2}.

The reactions were terminated after a 10-min incubation period at 25° by the addition of 0.3 ml of 20% trichloroacetic acid. The assay used for [^{32}P]ATP was identical to that given by AVRON[10] which removes all the non-esterified P_i into a non-aqueous phase (benzene–isobutanol). Aliquots of the aqueous phase, which contain only organic phosphate, were placed in planchets, dried, and counted on a Nuclear Chicago gas-flow detector (Model D47). The overall counting efficiency of the sample geometry and the detector was determined and used to convert counts/min into μmoles ATP esterified. Two types of controls were examined: one set of flasks contained the complete reaction mixture but received no light during the incubation period; a second set received light but lacked phenazine methosulphate. Both controls showed the same background activity and were therefore averaged before subtraction from the complete assays. The rates for ATP synthesis shown in Table III represent the net rates of light-dependent, phenazine methosulphate-catalyzed phosphorylation.

ATPase activity was assayed by the addition of approx. 1 μC of [^{32}P]ATP in place of the $^{32}P_i$ of the standard reaction mixture. A total of 0.25 μmole ATP per reaction mixture was added. The reaction mixtures were incubated for 10 min under conditions identical to those for photophosphorylation assays. ATPase activity was assayed by counting the radioactivity present in an aliquot of the first benzene–isobutanol extraction of the molybdate-treated reaction mixtures[10]. Dark controls were run for each assay.

Glucose assimilation

The algae were harvested by centrifugation and were washed once with the reaction medium (0.03 M potassium phosphate buffer (pH 6.8), 0.0185 M glucose). Five reaction vessels were prepared for each cell type; each vessel contained 100 μl packed cell volume, 10 mg glucose, and 0.25 μC uniformly labeled [^{14}C]glucose in 3 ml of 0.03 M potassium phosphate buffer (pH 6.8). The vessels were placed on a Gilson respirometer and gassed for 10 min with argon. Three vessels of each cell type were incubated in white light while two were kept in darkness. The light intensity was $1.5 \cdot 10^5$ ergs·sec^{-1}·cm^{-2}. Oxygen-free argon was continually passed through the reaction flasks. After 2 h incubation at 25° the reaction was terminated by centrifuging out the cells which were then washed twice with distilled water and finally boiled briefly in 80% ethanol. The insoluble portion was centrifuged into planchets (precipitate fraction, Table IV) and the supernatant poured off. Aliquots of the supernatant fraction (soluble fraction, Table IV) were also placed on planchets. Both sets were then dried and counted.

Nitrite reduction

25 μl packed cell volume of thoroughly washed cells in 2.5 ml of 0.05 M potassium phosphate buffer (pH 7.2) were placed in each of two Warburg flasks. $NaNO_2$

(3 μmoles in 0.5 ml) was added to the sidearm of each flask. The cells were adapted for photoreduction in a gas phase of 96 % H_2–4 % CO_2. Gas exchange was measured the following morning under the same gas phase.

RESULTS AND DISCUSSION

Chloroplast photoreductions

Three types of photoreductions may be obtained with chloroplast preparations: (1) those that require only System II activity; (2) those that require only System I activity; and (3) those that require both activities. Examination of these three types of photoreductions in chloroplasts isolated from photosynthetic mutants should provide a means for classifying the mutant organisms in relation to the two light reaction hypothesis of photosynthesis.

Table I summarizes the information obtained on these three classes of chloroplast photoreductions in the normal and mutant strains of Scenedesmus. The rates of photoreduction are expressed as μmoles oxidant reduced per mg chlorophyll per h. (Negligible rates of $NADP^+$ photoreduction are expressed as <5 μmoles per mg chlorophyll per h because such low rates are difficult to distinguish from background noise as well as from light-independent changes in $A_{340\ nm}$.) DCIP reduction represents Type (1) above, cytochrome *c* and $NADP^+$ reduction represent Type (3), and DCIP–ascorbate–$NADP^+$ reduction represents Type (2). Chloroplasts of the wild-

TABLE I

REDUCTIVE ACTIVITIES OF SCENEDESMUS CHLOROPLASTS

All reduction rates are expressed as μmoles oxidant reduced per mg chlorophyll per h. *A*. 0.12 μmole DCIP, 3 μmoles KCN, 40 μg chlorophyll, brought to 3.0 ml with 0.5 M sucrose, 0.05 M Tris–HCl buffer (pH 8.0), and 0.03 M KCl. *B*. 1 mg cytochrome *c*, saturating photosynthetic pyridine nucleotide reductase, 150 μmoles phosphate buffer (pH 6.7), 6 μmoles $MgCl_2$, and 40 μg chlorophyll, brought to 3.0 ml final volume with distilled water. *C*. Same as *B*, but 0.8 μmole $NADP^+$ in place of cytochrome *c*. *D*. Same as *B*, but 0.1 μmole DCIP, 10 μmoles sodium ascorbate, and 0.8 μmole $NADP^+$ in place of cytochrome *c*. Wild-type chloroplasts were assayed in the presence of $5 \cdot 10^{-6}$ M DCMU.

Chloroplasts (source)	*Reaction mixture*			
	A (DCIP)	*B (cytochrome c)*	*C ($NADP^+$)*	*D (DCIP–ascorbate–$NADP^+$)*
Wild type, fresh	61	117	52	31
frozen	42	98	35	20
Mutant 8, fresh	16	18	<5	<5
frozen	15	15	<5	<5
Mutant 11, fresh	0	2	<5	24
frozen	0	2	<5	21
Mutant 26, fresh	1	2	<5	16
frozen	3	2	<5	17
Mutant 40, fresh	2	7	<5	22
frozen	1	0	<5	18
Mutant 50, fresh	1	0	<5	14
frozen	1	3	<5	12
Mutant a′, fresh	0	1	<5	25
frozen	0	3	<5	27

type strain possess all three types of activity. The photoreductions of DCIP, cytochrome *c*, or $NADP^+$, which require water as the reductant, indicate the rate of electron flow as about 100 μequiv per mg chlorophyll per h. Freezing and thawing of the chloroplasts was found to have little effect on their activity.

Five of the six mutants were incapable of utilizing water as a reductant. However, these five mutants all possess System I activity, as indicated by their ability to reduce $NADP^+$ with DCIP–ascorbate as the reductant (Table I). The rates of DCIP–ascorbate–$NADP^+$ reduction obtained with chloroplasts from Mutants 11, 40, and a′ approximate that obtained with chloroplasts from wild-type cells. The inability of Mutants 26 and 50 to perform photoreduction, as well as observations on the 520-nm light-induced absorbance change which will be published elsewhere, suggest that the defects in these two mutants are located in a different portion of the photosynthetic apparatus than the defects in Mutants 11, 40, and a′. The somewhat lower rates of DCIP–ascorbate–$NADP^+$ photoreduction obtained with chloroplast preparations from Mutants 26 and 50 as compared to the wild-type strain and Mutants 11, 40, and a′ may result from the different locations of the defects in the two classes of mutants.

The data of Table I verify the earlier observation that Mutants 11, 40, and a′ are oxygen (System II) mutants[2]. The lack of cellular photoreduction by Mutants 26 and 50, as well as the apparent partial inhibition of System I photoreactions in these two mutants, suggest that their defects are located in the electron transport chain connecting Photosystem II to Photosystem I.

Of the mutants examined, only the chloroplasts of Mutant 8 possess Hill reaction activity (Tables I and II). These chloroplasts photoreduced DCIP and ferricyanide but not $NADP^+$, even when DCIP–ascorbate was the reductant. This finding verifies the earlier results of KOK AND DATKO[11] who also examined this mutant. DCIP photoreduction occurred at only about one-fourth to one-third the rate found for wild-type chloroplasts while ferricyanide reduction occurred at approximately one-half to four-fifths the wild-type rate (Table II). GORMAN AND LEVINE[12] have observed that both plastocyanin and cytochrome 553 are required for DCIP reduction by isolated chloroplasts of Chlamydomonas. Since plastocyanin and cytochrome 553 are kinetically close to the reactive center of System I (refs. 13, 14), it is possible that the depressed rate of DCIP reduction with chloroplasts from Mutant 8 is due

TABLE II

COMPARISON OF HILL REACTION ACTIVITIES IN WILD-TYPE AND MUTANT 8 CHLOROPLASTS

Rates of reduction are expressed as μmoles oxidant reduced per mg chlorophyll per h. Reaction mixtures are as described for DCIP in Table I except that 2 μmoles $K_3Fe(CN)_6$ are substituted for DCIP where indicated.

Chloroplasts (source)	*Hill oxidant*			
	Expt. 1		*Expt. 2*	
	$K_3Fe(CN)_6$	*DCIP*	$K_3Fe(CN)_6$	*DCIP*
Wild type	182	55	154	49
Mutant 8	85	15	125	15
% wild-type activity in Mutant 8	47	27	81	31

to a requirement for System I activity (or at least System I integrity) in order to obtain maximal rates. The slightly higher rates of reduction found with ferricyanide may be attributed to lesser dependence of the latter oxidant on System I for maximal reduction rates. The chloroplast photoreduction data of Mutant 8 are entirely consistent with the earlier findings on whole cell reactions (*p*-benzoquinone Hill reaction and photoreduction) which indicate that it is a System I mutant[2].

Table I indicates that chloroplasts from Mutant 8 will reduce cytochrome *c* at approx. 15% the rate obtained with chloroplasts from wild-type cells. Thus, it is suggested that System II alone may be capable of photoreducing cytochrome *c*, although at a much reduced rate over that obtained with both Systems I and II. The lower reducing potential generated by System II (refs. 11, 15) would account for the lower rate of cytochrome *c* reduction by Mutant 8. However, the reduction of cytochrome *c* by Mutant 8 chloroplast preparations could also be interpreted as an indication of residual System I activity which is not apparent in the $NADP^+$ photoreduction assay.

The photoreduction rates observed for chloroplasts isolated from Scenedesmus (Tables I and II) are low in comparison to those in the literature for other systems[16]. Numerous attempts were made to improve these rates by altering the constituents and the pH of the reaction media. No improvement could be obtained in this manner, and it was assumed that the relatively low rates obtained resulted from the fragmentation of the chloroplasts during the isolation procedure.

Spinach photosynthetic pyridine nucleotide reductase was generally used to stimulate all $NADP^+$ and cytochrome *c* photoreductions because photosynthetic pyridine nucleotide reductase from Scenedesmus, prepared according to the procedure of SAN PIETRO AND LANG[7], did not catalyze light-dependent $NADP^+$ reduction with Scenedesmus chloroplasts. However, light-dependent $NADP^+$ reduction was obtained with Scenedesmus chloroplasts in the presence of a 144000 $\times$ *g* supernatant of disrupted algal cells.

Photophosphorylation

Measurements for both cyclic and non-cyclic photophosphorylation were attempted with algal chloroplasts prepared by a variety of procedures. Non-cyclic photophosphorylation was never obtained, either with ferricyanide or $NADP^+$ as the oxidant. This failure to obtain a non-cyclic photophosphorylation does not necessarily suggest its absence in Scenedesmus chloroplasts, but more probably its relative lability to the extraction and assay procedure.

All chloroplasts, except those from Mutant 8, exhibited phenazine methosulphate-mediated cyclic photophosphorylation (Table III). Ascorbate, in preference to reduced glutathione, was necessary for activity. Since cyclic photophosphorylation is generally considered a System I photoreaction, these data support those obtained on chloroplast photoreductions. Mutant 8 was shown to be defective in Photosystem I (Table I) which agrees with the absence of cyclic photophosphorylation activity. It is of interest that Mutants 26 and 50 possess cyclic photophosphorylation activity even though they are apparently defective in the electron transport chain. It is evident that the site of re-entry of electrons into System I is more removed from Photosystem II than the site of the defects in Mutants 26 and 50.

The rates of photophosphorylation in cell-free preparations of Scenedesmus

(4–20 μmoles/mg chlorophyll per h) are extremely low in comparison with those for chloroplasts of spinach. AVRON AND SHAVIT[17] reported fixation rates of up to 1200 μmoles ATP per mg chlorophyll per h with preparations from spinach. We attempted one assay using chloroplasts from spinach and the rate obtained is presented in Table III. Although a rate of 90 μmoles per mg chlorophyll per h is still much lower

TABLE III

CYCLIC PHOTOPHOSPHORYLATION AND ATPase ACTIVITIES IN SCENEDESMUS CHLOROPLASTS

Photophosphorylation rates are expressed as μmoles ATP esterified per mg chlorophyll per h. Rates of ATP hydrolysis are expressed as μmoles ATP hydrolyzed per mg chlorophyll per h. Reaction conditions are given under METHODS.

Assay	*Chloroplasts (source)*							
	Wild type	*Mutant strain No.*						*Spinach*
		8	*11*	*26*	*40*	*50*	*a'*	
Phosphorylation								
1*	8	0	9	5	5	12	17	90
2*	15	0	8	4	20	11	16	
ATP hydrolysis								
1	1.2	1.2						1.4

* Assays 1 and 2 are independent and replicate experiments.

than those in the literature, it does indicate that our assay procedure is not the only reason for obtaining weakly active chloroplasts from Scenedesmus. The fragmentation of chloroplasts during the isolation procedure probably accounts in large part for the low activities obtained. KAHN[18] reported similar frustrations in attempts to obtain higher rates of photophosphorylation from isolated chloroplasts of *Euglena gracilis*.

ATPase activities with chloroplasts from wild-type Scenedesmus, Mutant 8, and spinach were assayed to determine whether the absence of photophosphorylation in Mutant 8 was a result of enhanced hydrolytic activity. As may be seen in Table III, this was not the case. The rate of hydrolysis by chloroplasts from Mutant 8 was no higher than that in wild-type or spinach preparations and was too low to account for the absence of cyclic photophosphorylation activity.

Glucose assimilation

Since whole cells of Mutants 26 and 50 perform neither Photosystem I nor II reactions, but isolated chloroplasts are capable of both cyclic photophosphorylation and photoreduction of $NADP^+$ with DCIP–ascorbate, attempts were made to measure *in vivo* photophosphorylation with these mutants. KANDLER AND TANNER[19] have proposed that the light-dependent, anaerobic assimilation of glucose represents a measurement of *in vivo* cyclic photophosphorylation. This process was therefore examined in Mutants 26 and 50 to test the hypothesis that the only function of the light reactions in the cellular process of photoreduction is the generation of ATP. The reducing potential required for CO_2 fixation would then be produced by non-photochemical reactions. This hypothesis is plausible since H_2 gas, *via* a hydrogenase, has sufficient reducing potential to reduce pyridine nucleotide without the assistance of photochemical energy. The data of Table IV show that neither mutant is capable

of light-dependent, anaerobic glucose assimilation. Thus, the hypothesis for photoreduction outlined above is neither supported nor rejected by these data. Unfortunately, the question as to the function of System I in photoreduction remains unanswered. It is apparent, however, that under our experimental conditions *in vitro* and *in vivo* cyclic photophosphorylation are not identical processes.

TABLE IV

ANAEROBIC GLUCOSE ASSIMILATION

The figures given are counts per min of radioactive glucose incorporated into the indicated fraction after a 2-h incubation at 25°.

Cells	*Fraction*	*Dark*	*Light*	*Light minus dark*
Wild type	Precipitate	4022	12674	8652
	Soluble	610	2119	1509
Mutant 8	Precipitate	4628	7277	2649
	Soluble	628	886	258
Mutant 26	Precipitate	3882	3756	−126
	Soluble	567	612	45
Mutant 50	Precipitate	2737	2382	−355
	Soluble	505	550	45

The rates of glucose assimilation presented in Table IV are expressed as counts/min since the combined counting efficiency of the detector and the sample geometry was not determined. If a counting efficiency of 10 % is assumed, the light-dependent glucose assimilation with wild-type cells is equivalent to a rate of glucose uptake of approx. 1 mg per h per 100 μl packed cell volume.

The data of Table IV indicate that Mutant 8 accumulated glucose at about 30 % of the wild-type rate. No explanation can be given for this light-stimulated uptake since Mutant 8 lacks both System I and *in vitro* cyclic photophosphorylation activities. It is also evident from Table IV that the assimilated glucose is quickly converted to an alcohol-insoluble form, presumably polysaccharide. A System II mutant (11) was also examined and was found to exhibit a rate of light-dependent glucose uptake equal to about one-half the wild-type rate confirming the results of TANNER, ZINECKER AND KANDLER[20].

Nitrite reduction

Non-photochemical nitrite reduction by chloroplasts may be used as an assay for hydrogenase activity[21]. Fig. 1 demonstrates that both Mutants 26 and 50 contain an active hydrogenase after adaptation in an atmosphere of 96 % H_2 and 4 % CO_2. Mutant 50 exhibits a rate of hydrogen consumption similar to that of wild-type cells while Mutant 26 has an enhanced rate almost double that of wild type. The inability of Mutants 26 and 50 to perform photoreduction is therefore not due to a deficiency of the hydrogenase itself.

CONCLUSION

Fig. 2 is a generalized scheme for the two light reaction series hypothesis of photosynthesis in which the portion of the photosynthetic apparatus defective in each

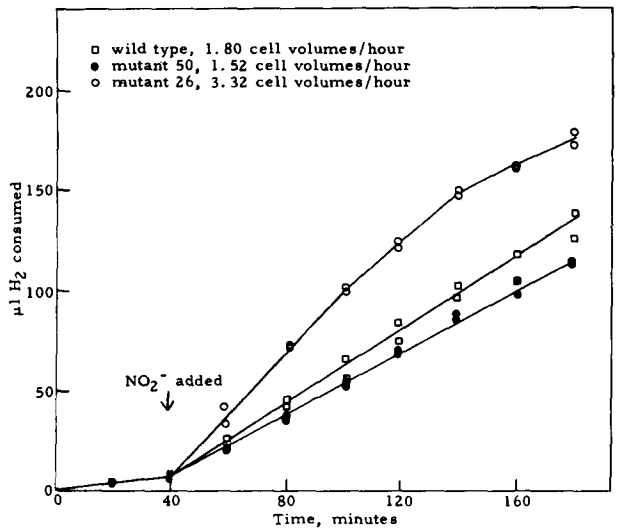

Fig. 1. Time course of nitrite reduction in wild-type Scenedesmus and in Mutants 26 and 50. $NaNO_2$ was added at time = 40 min. The rates of gas uptake are corrected for those prior to the addition of $NaNO_2$. The initial linear rate is given for Mutant 26.

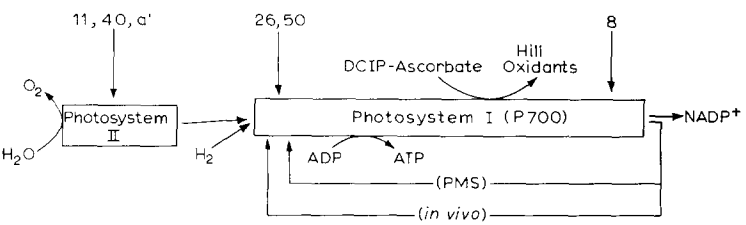

Fig. 2. Location of several mutants of Scenedesmus in a generalized version of the two light reaction series formulation for photosynthesis. PMS, phenazine methosulphate.

of the six mutants studied is indicated. Mutant 8 had earlier been shown to be lacking P 700 (ref. 2) and evidence presented here verifies its status as a System I mutant. Both cellular and chloroplast reactions indicate that Mutants 11, 40, and a' are defective close to the site of O_2 evolution (Photosystem II) as indicated in Fig. 2. Mutants 26 and 50, however, were shown to be blocked between Photosystem II and the site of phosphorylation in the electron transport chain. Results from glucose assimilation experiments further indicate that the defects are beyond the site where electrons from cyclic photophosphorylation *in vivo* enter the electron transport chain. The defects of these two mutants are consequently placed between the two photosystems as indicated in Fig. 2.

Examination of the absorption spectra *in vivo* of the System II and electron transport mutants and wild-type cells showed that no significant differences existed*. Previous examination of Mutant 8 revealed that its absorption spectrum *in vivo* lacks a shoulder at 700 nm (ref. 22).

Mutant 8 consistently demonstrates a much reduced System II activity in com-

* W. L. BUTLER, personal communication.

parison with that obtained with wild-type cells, both in the *p*-benzoquinone Hill reaction and chloroplast photoreductions (Tables I and II)[11]. Also, Mutants 26 and 50, which are defective in the electron transport chain, apparently exhibit decreased System I activity, both in DCIP–ascorbate–$NADP^+$ photoreduction (Table I) and in the 520-nm light-induced absorbance change. (The latter observations will be reported in a later paper.) It is suggested that all three are defective in one or more components of 'Photosystem I', where Photosystem I is now defined as indicated by the large, enclosed area in Fig. 2. Supposedly, all of the various reactions indicated (DCIP–ascorbate–$NADP^+$ reduction, Hill reactions, photophosphorylation, and photoreduction) involve the same physical unit, although the different cofactors interact at different locations along that physical entity. Consequently, defects anywhere within System I, as defined by Fig. 2, would be expected to disrupt the functioning of the entire system with the result that those reactions most closely associated with the defect would be entirely absent while others less closely associated with the defect would be inhibited by an intermediate amount.

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