*Biochimica et Biophysica Acta*, 430 (1976) 105–112


BBA 47058

# LIGHT-DEPENDENT DEVELOPMENT OF PHOTOSYNTHETIC COMPETENCE IN *SCENEDESMUS* MUTANT No. 8

HANS J. RURAINSKI and HANS J. HOPPE

*Lehrstuhl für Biochemie der Pflanze der Universität, 3400 Göttingen, Untere Karspüle 2 (G.F.R.)*

(Received August 26th, 1975)

## SUMMARY

The heterotrophically grown, *P*-700-free mutant No. 8 of *Scenedesmus obliquus* is unable to carry out photosynthesis. Yet, chloroplast particles isolated from the alga reduced ferricyanide. They also reduced methyl viologen in the presence of the artificial donor reduced 2,6-dichlorophenol indophenol with a low yield but an appreciable saturation rate. NADP reduction or *P*-700 turn-over could not be detected.

When grown mixotrophically, the mutant showed increasing *P*-700 activity with a concomitant increase in the rate of photosynthesis. Both activities were lost again when the algae were returned to darkness. Isolated chloroplast particles showed a good *P*-700 turn-over and reasonable rates of NADP reduction.

The data suggest that the mutation occurred at a site preceding the formation of the pigment. The results on the photochemical activities are discussed in the light of reports concerning the involvement of *P*-700 in linear electron transport.

## INTRODUCTION

Mutant strains of several algae have long been investigated in an attempt to elucidate various aspects of the mechanism of photosynthesis (for a recent review, see ref. 1). Of these, the *P*-700-free mutant No. 8 of *Scenedesmus obliquus* was of particular interest because of contradictory results bearing on the involvement of *P*-700 in the linear photosynthetic electron transport leading to the reduction of NADP (for a recent review see ref. 2).

Thus, Kok and Datko [3] and Pratt and Bishop [4] showed that chloroplast particles of the mutant did not reduce NADP with either water or reduced 2,6-dichlorophenol indophenol ($DCPIPH_2$) as electron donor. In contrast, Gee et al. [5] reported that such particles could photoreduce NADP in the presence of $DCPIPH_2$ at rates comparable to those obtained with wild type preparations. Using pea chloroplasts or intact algae Rurainski and co-workers [6, 7] found antagonistic relationships

Abbreviations: NADP, nicotinamide adenine dinucleotide phosphate; DCPIP, 2,6-dichlorophenol indophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

between the rate of electron transport through *P*-700 and NADP reduction or oxygen evolution. In the presence of $Mg^{2+}$, for example, the yield of *P*-700 was insignificant compared to the yield of NADP reduction. These contradictions and the fact that improved procedures for the isolation of chloroplast particles from *Scenedesmus* have recently been reported [8] led us to reinvestigate the problem.

In the course of the work we observed that the mutant developed the ability to evolve oxygen concomitant with appreciable *P*-700 activity when grown mixotrophically [5]. Upon returning the light-grown cells to darkness, the mutant characteristic reappeared. Experiments with intact cells and isolated chloroplast particles will be reported.

## MATERIALS AND METHODS

*S. obliquus* strain $D_3$ and mutant No. 8 were grown in a medium described by Bishop and Senger [9] except that sodium citrate was omitted and $Fe_2(SO_4)_3$ was complexed with $6 \cdot 10^{-5}$ M EDTA. For heterotrophic and mixotrophic growth, the medium also contained 0.5 % glucose and 0.25 % yeast extract and the temperature was 21 °C. The light intensity was 18 000 lux.

Chloroplast particles were isolated from cells harvested in the logarithmic phase of growth (wild type, autotrophic after 24–30 h; wild type heterotrophic and mutant after 4–5 days) according to the method of Berzborn and Bishop [8]. The particle yield on a chlorophyll basis was between 25 and 50 %. Chlorophyll concentrations were determined after MacKinney [10]. Ferredoxin and ferredoxin-NADP-oxidoreductase were isolated according to Buchanan and Arnon [11] and Shin [12]. Rates of ferricyanide and NADP reduction were measured spectroscopically at 420 or 340 nm, respectively. Red actinic light was obtained with a RG 610 glass filter (Schott); the intensity was $1.6 \cdot 10^6$ ergs $\cdot$ cm$^{-2} \cdot$ s$^{-1}$. *P*-700 was measured at 700 nm with a relaxation spectrometer described previously [7, 13] using red actinic light of intensity $1.6 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s$^{-1}$. Oxygen uptake and evolution were followed with a Clark-type Pt-AgCl electrode (Rank Bros.) at a temperature of 25 °C.

## RESULTS

As has been shown previously for whole cells of *Chlorella* and *Anacystis* there exists an antagonistic relationship between oxygen evolution and the electron transport rate through *P*-700 in the presence of low concentrations of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) [7, 14]. Fig. 1 shows the same effect with whole cells of wild type *Scenedesmus*. As the rate of oxygen evolution decreases, the flux through *P*-700 increases. With approx. $2 \cdot 10^{-7}$ M DCMU, a maximum is reached followed by an inhibition. The relaxation time of the pigment remains reasonably constant at about 18 ms. These data indicate that in order to obtain maximum *P*-700 turn-over in intact cells, photosynthesis must be partially inhibited. Therefore, in the following experiments we have measured *P*-700 activity under this condition.

The rate of oxygen evolution and *P*-700 flux of mixotrophically grown *Scenedesmus* mutant No. 8 are shown in Fig. 2. At zero time we are dealing with the heterotrophic organism lacking oxygen evolution and showing only a small apparent *P*-700 signal. (For an explanation of the latter measurement see discusison of Fig. 3).

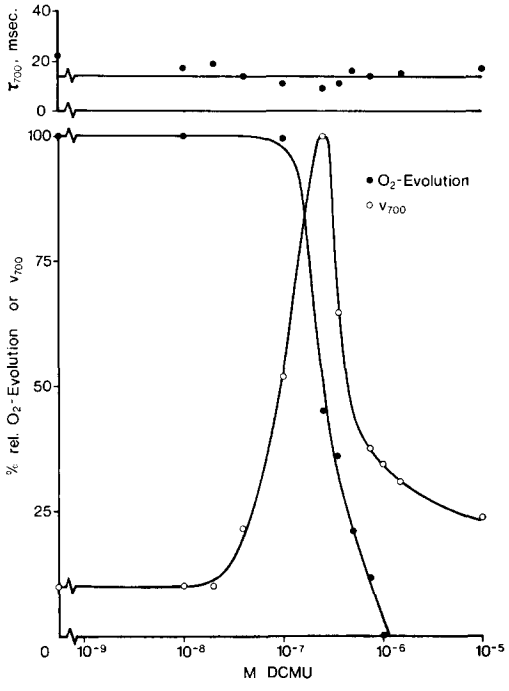


Fig. 1. Relative rates of oxygen evolution and *P*-700 flux in intact cells of wild type *Scenedesmus* as a function of DCMU concentration. The reactions were carried out in the growth medium. The maximum oxygen evolution rate was 147, the maximum flux 139 $\mu$mol/mg chlorophyll per h. The data were normalized to these values. Modulation frequency for *P*-700 measurements was 7.2 Hz.

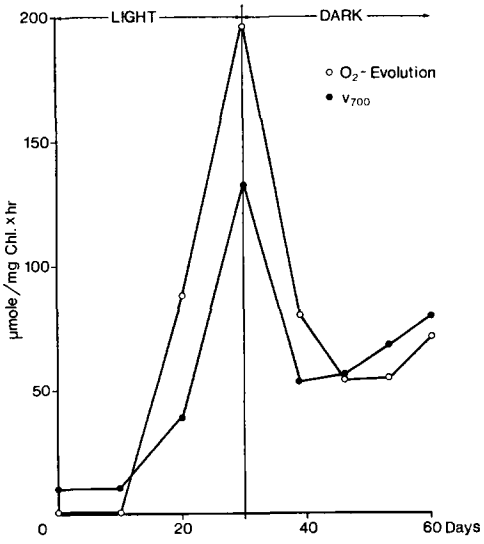


Fig. 2. Rates of oxygen evolution and *P*-700 flux in intact cells of mixotrophically grown mutant No. 8. Reaction conditions as in Fig. 1. *P*-700 was measured in the presence of $2 \cdot 10^{-7}$ M DCMU.

The cultures were then illuminated with continuous white light (approx. 18 000 lux) for the number of days indicated and during this time transferred to fresh medium every 5 days. After a lag of about 10 days the rate of oxygen evolution and the flux through *P*-700 increased reaching their maximum observed values after 30 days. At this stage, the cells grew in the light on minimal medium and at about the same rate as the wild type. Thus, these organisms appeared fully competent for autotrophic growth [5].

Upon returning such cultures to darkness, both the ability to evolve oxygen and the *P*-700 activity decreased considerably, reaching in some experiments after 30 days, values much lower than indicated in Fig. 2. Wild type cells grown under the same conditions showed only slight and random variations over the same period, exhibiting oxygen evolution rates of 144 and a *P*-700 flux of 67 μmol/mg chlorophyll per h. This observation makes it appear unlikely that the observed results are due to a contamination of the cultures with wild type cells.

Fig. 3 shows absorption changes in the red region of the spectrum. At the beginning of mixotrophic growth and after 10 days in the light, an absorption band with a maximum at 685 nm was observed. The relaxation time was between 15 and 18 ms. The tail of this band extends to 700 nm and is responsible for the small apparent flux shown in Fig. 2. An absorption spectrum typical for *P*-700 developed only in the

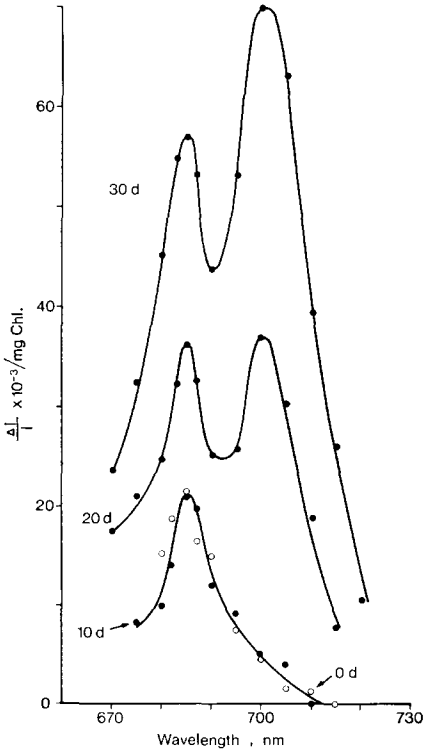


Fig. 3. Difference spectra of mixotrophically grown mutant No. 8 as a function of time in the light. Reaction conditions as in Fig. 1. The measurements were made in the presence of $2 \cdot 10^{-7}$ M DCMU.

TABLE I

*P*-700 FLUXES IN CHLOROPLAST PARTICLES OF *S. OBLIQUUS*

The reaction mixture contained 130 mM sucrose, 20 mM tricine (pH 7.8), 20 mM KCl, 5mM $MgCl_2$ and 2 mM $NH_4Cl$. Substrates: 0.25 mM NADP (with saturating amounts of ferredoxin and ferredoxin-NADP-oxidoreductase) or 0.8 mM ferricyanide or 1 mM methyl viologen; donor system: 1 $\mu$M DCMU, 0.1 mM DCPIP, 1 mM sodium ascorbate. Modulation frequency, 7.5 Hz. All fluxes expressed as $\mu$equiv./mg chlorophyll per h.

| System | Wild type (autotrophic) | Mutant No. 8 (heterotrophic) | Mutant No. 8 (mixotrophic) |
|---|---|---|---|
| Water → FeCN | 30.5 | 0 | 18.0 |
| Water → NADP | 59.5 | 0 | 14.1 |
| $DCPIPH_2$ → NADP | 77.8 | 0 | 64.3 |
| $DCPIPH_2$ → methyl viologen | 85.9 | 0 | 75.6 |

course of continued mixotrophic growth. We have not investigated the 685 nm pigment further. It is possible, however, that it is related to an absorption band described by van Gorkom et al. [15], since the wavelength of maximal absorption and the half-band width are similar.

To further characterize the effect of the light, we isolated chloroplast particles from cells grown under different conditions and measured *P*-700 fluxes and terminal reactions with a number of electron acceptors. *P*-700 fluxes are listed in Table I. Wild type preparations showed generally higher values with the artificial electron donor $DCPIPH_2$ than with water. Mutant particles were devoid of any *P*-700 activity. The pigment with maximum absorbance at 685 nm described above was also present here, but the signal due to its end absorption at 700 nm has been subtracted from these data. Particles isolated from the mixotrophically grown cells showed again good *P*-700 fluxes which in the case of the donor system were of the same magnitude as the wild type.

Rates of photoreductions associated with the various particles are shown in Table II. Preparations of the wild type and mixotrophically grown mutant carried out all reactions tested with reasonable rates, although the rate of NADP reduction in

TABLE II

PHOTOREDUCTION OF VARIOUS ACCEPTORS BY CHLOROPLAST PARTICLES OF *S. OBLIQUUS*

Reaction conditions as in Table I, except that the actinic light was of saturating intensity. All rates expressed as $\mu$equiv./mg chlorophyll per h.

| System | Wild type (autotrophic) | Mutant (heterotrophic) | Mutant No. 8 (mixotrophic) |
|---|---|---|---|
| Water → FeCN | 203.8 | 120.0 | 81.0 |
| Water → NADP | 147 | 0 | 41.4 |
| $DCPIPH_2$ → NADP | 88.4 | 0 | 24.7 |
| $DCPIPH_2$ → methyl viologen | 712.0 | 282.0 | 331.0 |

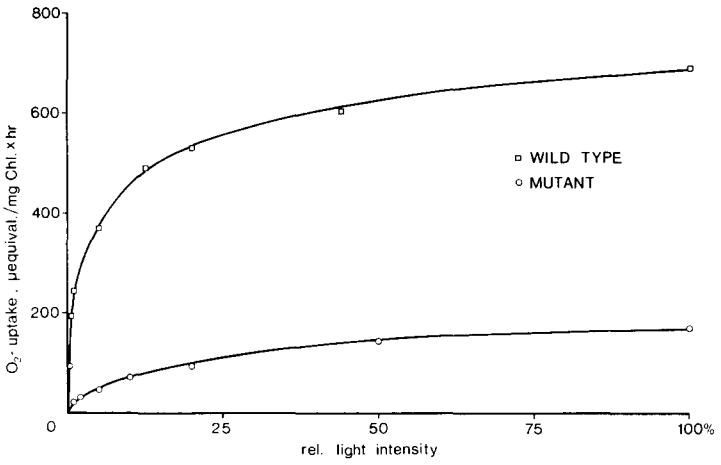


Fig. 4. Oxygen uptake as a function of light intensity in chloroplast particles of the wild type and mutant No. 8. Substrate: methyl viologen. Reaction conditions as in Table I, donor system; 20 $\mu$g chlorophyll/ml. The maximum intensity was $10^6$ ergs · $cm^{-2}$ · $s^{-1}$.

wild type particles was somewhat below that reported by Berzborn and Bishop [8]. In agreement with several reports (e.g. refs. 3 and 4) but in contrast to Gee et al. [5] mutant particles could not photoreduce NADP with either water or $DCPIPH_2$ as electron donor. They did, however, reduce ferricyanide albeit with lower saturation rates than wild type preparations. A possible explanation for this observation is that in wild type particles both photosystems contribute to the reduction while in mutant particles only Photosystem II is active [2].

Despite the lack of NADP reduction, mutant particles showed an appreciable rate of methyl viologen reduction with an artificial electron donor. Since according to present theory (see ref. 2) methyl viologen is reduced by the low potential primary reductant of Photosystem I at the same site as NADP, this result was unexpected. Fig. 4 indicates that the relative quantum yield of this reaction is quite low compared to the wild type. From the initial slopes of the light intensity curves, we estimated the differences to be a factor of 40. However, the saturation rates were appreciable, varying between one-third and one-half of the wild type rates. Similar results for mutant particles were reported for the reaction water → methyl viologen [3].

## DISCUSSION

The data reported here bear on two problems, the first of which concerns the mutation of *Scenedesmus* No. 8. Clearly, the mutant characteristics are expressed only in the dark. When grown on organic medium and in light for extended periods, the organism shows properties of the wild type. That this result is not due to a contamination of the cultures by the wild type is indicated by the observation that the light-grown mutant, in contrast to the wild type, looses its acquired photosynthetic capacity when returned to darkness. This observation also excludes the possibility of a genetic reversion of the mutation. The long time required for this action of the light is rather

puzzling since other effects such as photoadaptation or photomorphogenesis to which these observations could be related take place in a matter of hours. Such a long time course is conceivable, if one assumes that only a very small number of mutant cells out of the population responds to the light. Given that these few cells have a higher growth rate than the majority, they would have a selection advantage and become enriched. *P*-700 is believed to be a chlorophyll molecule complexed with a protein [1]. Since the gross chlorophyll composition of the wild type, mutant and light-grown mutant was quite similar the data suggest that either the protein is only being synthesized in the light or that the protein is present and the light served to form the complex. Other mechanisms are possible and further work is necessary to establish the causes for the action of the light. At any rate, the experiments indicate that the mutation has affected a site preceding the formation of the pigment rather than the pigment itself.

The data also bear upon the question of the involvement of *P*-700 in linear electron transport. As Rurainski and co-workers [6, 7] have shown, *P*-700 reacting with a relaxation time of about 20 ms was in several instances inversely related to NADP reduction or oxygen evolution. Fig. 1 of this communication is a further example. The authors challenged the assumption that the 20-ms component participated in linear electron transport but they could not rigorously exclude that a faster reacting component which may have excaped the measurement was so involved. The report of Gee et al. [5] showing rates of NADP reduction with mutant particles which were as high as those with wild type preparations seemed to support this idea, although these authors interpreted their data by assuming a direct interaction of NADP with the primary reductant of Photosystem II.

We have made considerable effort to reproduce the results of Gee et al. [5]. But despite careful adherence to the (different) particle isolation procedure, the addition of raw enzyme extracts from chloroplasts in addition to ferredoxin and ferredoxin-NADP-reductase and changes in the pH of the reaction medium, the results were always negative. Also the observation of Gee et al. [5] that an ESR signal due to *P*-700 appeared in the mutant cells when they were heated and treated with detergents could not be reproduced by spectroscopy.

In this connection, the data on the reduction of methyl viologen with mutant particles are surprising. Since Photosystem II was inhibited in these experiments and Photosystem I ostensibly lacking, no reaction should occur. Kok and Datko [3] who found similar results with water as an electron donor suggested that this reaction could be due to "a remnant of normal or adulterated Photosystem I". If this were the case, the reduction of NADP could require an additional unknown component which is unnecessary for methyl viologen reduction. Alternatively, the reduction of methyl viologen under these conditions may proceed without an intact electron transport system including *P*-700. In the light of these and other [3] experiments the usual conclusion that this reaction is an indicator of Photosystem I activity should be examined critically.

As Bishop [1] pointed out in his detailed discussion of data obtained with *Scenedesmus* No. 8, the lack of photosynthesis in mutant cells and of NADP reduction in mutant particles argues for a role of *P*-700 in electron transport and against the proposal of Rurainski and co-workers [6, 7]. Certainly, the correlation between *P*-700 and $O_2$ evolution and/or NADP reduction shown here suggests that the pres-

ence of the pigment is required for both reactions. We feel, however, that such results alone are insufficient as strict evidence for a direct and dynamic participation of the pigment in photosynthetic electron transport. As other authors [6, 7, 14] have shown and as we show in Fig. 1, a *P*-700 component turning over in approx. 20 ms can be inversely related to photosynthesis or NADP reduction in both intact cells and chloroplasts of higher plants. In the light of these data we must assume that perhaps a *P*-700 component exists which reacts too fast for detection with our and most other spectrophotometric methods presently used in the field.

ACKNOWLEDGEMENTS

The authors thank Mr. Gerhard Mader for excellent technical assistance. This work was financially supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

1 Bishop, N. I. (1973) Current Topics in Photobiology and Photochemistry, Photophysiology, Vol. VIII, pp. 65–96, Academic Press, New York
2 Trebst, A. (1974) Ann. Rev. Plant Physiol. 25, 423–458
3 Kok, B. and Datko, A. (1965) Plant Physiol. 40, 1171–1177
4 Pratt, L. H. and Bishop, N. I. (1968) Biochim. Biophys. Acta 153, 664–674
5 Gee, R., Saltman, P. and Weaver, E. (1969) Biochim. Biophys. Acta 189, 106–115
6 Rurainski, H. J., Randles, J. and Hoch, G. (1971) FEBS Lett. 13, 98–100
7 Rurainski, H. J. (1975) Z. Naturforsch. 30c, 761–770
8 Berzborn, R. J. and Bishop, N. I. (1973) Biochim. Biophys. Acta 292, 700–714
9 Bishop, N. I. and Senger, H. (1971) in Methods in Enzymology (San Pietro, A., ed.), Vol. 23, pp. 53–56, Academic Press, New York
10 MacKinney, G. (1941) J. Biol. Chem. 140, 315–322
11 Buchanan, B. B. and Arnon, D. I. (1971) in Methods in Enzymology (San Pietro, A., ed.), Vol. 23, pp. 413–440, Academic Press, New York
12 Shin, M. (1971) in Methods of Enzymology (San Pietro, A., ed.), Vol. 23, pp. 440–447, Academic Press, New York
13 Hoch, G. E. (1971) in Methods of Enzymology (San Pietro, A., ed.), Vol. 23, pp. 297–303, Academic Press, New York
14 Kok, B. and Hoch, G. E. (1961) in Light and Life (McElroy, A. and Glass, B., eds.), pp. 397–416, The Johns Hopkins Press, Baltimore
15 Van Gorkom, H. J., Tamminga, J. J. and Haveman, J. (1974) Biochim. Biophys. Acta 347, 417–438