Biochimica et Biophysica Acta, 1101 (1992) 353–358
© 1992 Elsevier Science Publishers B.V. All rights reserved 0005-2728/92/$05.00

BBABIO 43639

Chlorophyll *a* fluorescence decay in herbicide-resistant D1 mutants of *Chlamydomonas reinhardtii* and the formate effect

Govindjee [a], Peter Eggenberg [b], Klaus Pfister [c] and Reto J. Strasser [b]

[a] *Departments of Physiology and Biophysics and Plant Biology, University of Illinois at Urbana, Champaign, IL (USA),* [b] *Laboratoire de Bioénergétique, Station de Botanique, Université de Genève, Lullier-Jussy (Switzerland) and* [c] *Agrochemical Division, Ciba-Geigy Basel (Switzerland)*

(Received 20 January 1992)
(Revised manuscript 6 April 1992)

Key words: Chlorophyll fluorescence decay; Photosystem II; Electron acceptor complex; Herbicide resistance; D1 mutant; (*Chlamydomonas reinhardtii*)

Intact cells of herbicide-resistant mutants of *Chlamydomonas reinhardtii*, altered in single amino acids in the D1 protein, were analyzed for chlorophyll *a* (Chl *a*) fluorescence decay in terms of electron transfer at the Q_AQ_B complex of Photosystem II. The D1 mutants Ar-207 (F255Y), Br-202 (L275F) and Dr-18 (V219I) had an almost unimpaired forward electron transfer ($Q_A^-Q_B^{(-)} \rightarrow Q_AQ_B^{(-)}$) and an almost unchanged ratio (0.1–0.2) of the slow to the fast PSII centers, compared to that of the wild type. Thus, the Q_B binding pocket was minimally modified. However, the mutants Ar-204 (G256D) and DCMU-4 (S264A) had an altered forward electron transfer, an altered apparent equilibrum for $Q_A^-Q_B \rightleftharpoons Q_AQ_B^-$ reaction, and an abnormally high ratio (0.6–0.8) of the slow to the fast PSII centers. These results suggest that these two mutations modified the Q_B binding pocket significantly. Addition of 100 or 200 mM formate led to an increase in the lifetime of the forward (Q_A^- to Q_B) electron transfer in the wild type, and the five mutants, with the largest change in the S264A, and the smallest in the L275F mutant. Formate treatment also led to a decrease in the apparent equilibrium for $Q_A^-Q_B \rightleftharpoons Q_AQ_B^-$ reaction and an increase in the fraction of the slow centers. All effects were reversed by 20 mM bicarbonate. We suggest that the amino acid S264, but not L275, F255 and V219, plays an important role in the binding and the function of plastoquinone and bicarbonate in PSII. The role of G256 remains to be further examined since glycine was changed to a significantly different amino acid, aspartic acid.

Introduction

Chlorophyll *a* (Chl *a*) fluorescence yield decay, after a saturating single-turnover flash, has been used to monitor electron transfer from Q_A^- to Q_B or Q_B^- in Photosystem II (PS II) [1–3] since Chl *a* fluorescence yield is high when $[Q_A^-]$ is high or when $[Q_A]$ is low [4]. These measurements and their analyses provide information on the functioning of the electron acceptor side of the reaction center II proteins D1 and D2 since Q_B and Q_A are located on these two proteins [5]. (For a recent review on the electron acceptor side of PS II, see Diner et al. [6].) In several D1 mutants of *Chlamydomonas reinhardtii*, the alteration of a single amino acid has been characterized by molecular biological techniques [7]. In addition to affecting herbicide binding, these structural alterations also modify, to a different degree, certain functional properties of PSII [7], including sensitivity to bicarb[illegible]ersible formate effect on Chl *a* fluorescen[illegible]]. The kinetics of Chl *a* fluorescence yiel[illegible] its decay after a single turnover flash, prov[illegible] an excellent tool for studying modified functional properties of the D1 protein, as compared to normal D1 of the wild type.

The earliest results on Chl *a* fluorescence yield decay, monitoring electron transfer from Q_A^- to Q_B, for a herbicide-resistant S264G mutant of a higher plant, suggested that this reaction was an order of magnitude slower in the mutant than in the wild type [9,10]. However, it was later shown [11–13], by other techniques, that there was an increased quasi-steady state concentration of Q_A^-, due to a decreased appar-

Correspondence to: Govindjee, Department of Plant Biology, University of Illinois at Urbana-Champaign, 265 Morrill Hall, 505 South Goodwin Avenue, Urbana, IL 61801-3707, USA.

Abbreviations: Mutants were named as follows: name of the wild type amino acid in single letter code, followed by the number of the amino acid and then the single letter code of the mutated amino acid.

ent equilibrium constant between $Q_A^- Q_B$ and $Q_A Q_B^-$. Furthermore, the newer analysis by Jansen and Pfister [14] has shown remarkable consistency in the half-decay time ($t_{1/2}$) of the fast phase of fluorescence ($t_{1/2}$, 314 ± 46 μs) among 28 different species of various types of plants (C_3, C_4 and CAM); however, five species of triazine-resistant plants had a 3-times slower (946 ± 100 μs) half-decay time of this phase of fluorescence.

Erickson et al. [7] reported data on the decay of the fast component of the fluorescence yield in several herbicide-resistant D1 mutants of *Chlamydomonas reinhardtii*, suggesting that in G256D and S264A mutants the half-time of Q_A^- decay was several times larger ($t_{1/2}$ > 1000 μs), whereas in L275F, F255Y and V219I mutants there was no effect ($t_{1/2}$, 300–400 μs) when compared to the wild type. In this analysis the ms (intermediate) and the s (slow) components were not included and, thus, no distinction was made between true changes in the reoxidation of Q_A^- from those in the apparent equilibrium constant. Etienne et al. [15] examined several herbicide-resistant D1 mutants of cyanobacteria *Synechocystis* 6714 and 7942, and one mutant (D1-S264A) of *Chlamydomonas reinhardtii*. On the basis of an analysis of the fluorescence yield decay into three components (cf. Ref. 16) and several other measurements, Etienne et al. concluded that in several cyanobacterial mutants the initial fast phase of the electron transfer from Q_A^- to Q_B was unaltered, but the electron transfer equilibrium was displaced toward Q_A^-. However, in D1-S264A of *C. reinhardtii*, the electron transfer lifetime was increased.

In this paper, we have analyzed the Chl *a* fluorescence yield decay in the wild type and five D1 mutants of *C. reinhardtii* (V219I, F255Y, G256D, S264A and L275F) in terms of three exponential decays, as in Refs. 15 and 16. It appears that amino acids V219, F255 and L275 are of marginal importance for the electron transfer from Q_A^- to Q_B. However, in G256D and S264A the fraction of the slow centers increases significantly; the lifetime of the forward electron flow from Q_A^- to Q_B increases; and the apparent equilibrium constant for $Q_A^- Q_B \rightleftharpoons Q_A Q_B^-$ reaction decreases. Since a bicarbonate-reversible formate inhibition occurs at the $Q_A Q_B$ complex [3,6] and various D1 mutants are differentially sensitive to it [8], we have included here an analysis of the chlorophyll *a* fluorescence decay in formate- and bicarbonate-treated D1 mutants of *C. reinhardtii*.

Material and Methods

Wild type *Chlamydomonas reinhardtii* cells and five herbicide-resistant D1 mutants: Dr-18 (V219I); Ar-207 (F255Y); Ar-204 (G256D); DCMU-4 (S264A); and Br-202 (L275Y), were grown autotrophically in a Tris-phosphate medium [8,17] for 2 days at 25°C (day) and 22°C (night). Cultures were illuminated for 16 h with fluorescent white light and kept in darkness for 8 h.

The decay of Chl *a* fluorescence yield was measured, as described earlier [14,18]. A 2 J 10 μs saturating xenon flash (EG and FY 611, Salem, MA), filtered through a Calflex cyan II (Balzers, Leichtenstein) and a BG 28 (Schott, Duryea, PA) glass filter, was used to convert all Q_A to Q_A^-. The fluorescence yield was monitored with a low-intensity (weak) measuring beam (660 nm), obtained from a pulsed light emitting diode (PAM 101 fluorimeter, Walz, Effeltrich, Germany), modulated at a frequency of 100 kHz. The fluorescence signal was detected using a solid state photodiode after it was passed through a long pass filter (> 700 nm) [18].

In all the samples (dilute suspensions of algal cells), fluorescence decay after an actinic flash was recorded (dark time between flashes, 1 s). Data from 16 recordings were averaged. The dark interval between the recordings was 10 s for the first flash data and 30 s for the third flash data. In intact cells, the ratio of Q_B to Q_B^- in darkness is about 1 (see e.g. Ref. 19) and, thus, the first and the second flash gave almost identical results (data not shown). As the data after the third flash were somewhat more stable, we report mostly these data.

Chl *a* fluorescence decay curves (4096 data points) were first stored in a digital Nicolet Explorer III (Madison, WI) oscilloscope. These data were transferred to a Hewlett-Packard series 200 computer and subsequently stored as ASCII files, which were used in a IBM-compatible personal computer for analysis. All data processing was done using BASIC programs developed by us (PE and RJS). Final curve fitting was made using the commercial non-linear data analysis program ENZFITTER [20].

The F_0, the minimum Chl *a* fluorescence yield, was obtained before the actinic flash was given. The fluorescence detector was electronically gated off during the actinic flash. The zero time for the fluorescence decay measurement was fixed at the time the actinic flash reached its maximum intensity. The first data point used in our measurement and analysis was at 170 μs, in order to avoid any possible gating and other artifacts.

For curve fitting the data sets were reduced to 250 data points using a smoothing program with a logarithmic time scale; there were more points at short and less points at long time intervals in order to determine precisely the parameters for the fast components. For representation, all curves were normalized to the fitted $F_{max} - F_0$.

Analysis of fluorescence decay as well as (Q_A^-) decay (assuming $p = 0.5$ [21]) curves into three components [15,16] are presented.

Formate/bicarbonate treatment was made by incubating the cells with 100 or 200 mM sodium formate and then 25 mM sodium bicarbonate for at least 10 min in darkness prior to measurements.

Results and Discussion

Chl *a* fluorescence yield decays with, at least, triphasic kinetics (see e.g., Refs. 15,16). The slow kinetics with a lifetime in the 1–2 s range, has been suggested to be due mainly to a back-reaction of Q_A^- with the oxygen-evolving state S_2, in the centers in which Q_A is not connected to Q_B and the PQ pool (see e.g., Ref. 15). Thus, it could include the kinetics of the inactive (non-Q_B) PSII centers [22,23] when present. On the other hand, the fluorescence yield decay in the sub-ms time scale, after a single turnover flash, reflects the kinetics of reoxidation of Q_A^- by Q_B (after the first flash) or by Q_B^- (after the second flash) when Q_B predominates in dark-adapted samples (see e.g. Ref. 25). In the ms time scale the variable Chl *a* fluorescence reflects the variable $[Q_A^-]$, which is in equilibrium with Q_B^- (see, e.g., Crofts and Wraight [24]).

Q_A^- decay curves and calculated parameters

Fig. 1 shows fluorescence decay curves, monitoring $[Q_A^-]$, for the wild type and four mutants (L275F, F255Y, S264A and G256D) after the third flash. The data points, and the best fits of three decay components to the data, are shown in the figure. Calculated parameters (amplitudes and lifetimes) are shown in Table 1. The major effects, or the absence of the effects, of the mutations are obvious: V219I, F255Y and L275F are very similar to the wild type. Thus, these amino acids may be of marginal importance for the electron transfer from Q_A^- to $Q_B^{(-)}$ and for the Q_B binding. However, in the G256D and S264A mutants both the fast and the intermediate fluorescence components are slower and the amplitude of the slow component is larger than that in the wild type.

In intact cells, the ratio of Q_B to Q_B^-, prior to the flash, is about 1, and thus an average lifetime for $Q_A^-Q_B$ to $Q_AQ_B^-$ and $Q_A^-Q_B$ to $Q_AQ_B^{2-}$ is monitored after almost all the flashes (see e.g. Ref. 19). Although the calculated lifetime of the fast decay component (τ_{fast}) does not directly monitor only the forward reactions, its value relfects this process. Although there is no significant difference in τ_{fast} among most of the mutants for both fluorescence and $[Q_A^-]$ decays, it is 2–3-fold larger for S264A than the wild type. Futhermore, there is a hierarchy (values ± 0.2 ms) in $\tau_{intermediate}$: WT ~ F255Y < V219I < L275 < S264A ~ G256D for both fluorescence and $[Q_A^-]$ decays. The τ_{int} of the S264A and G256D mutants were larger by 3- to

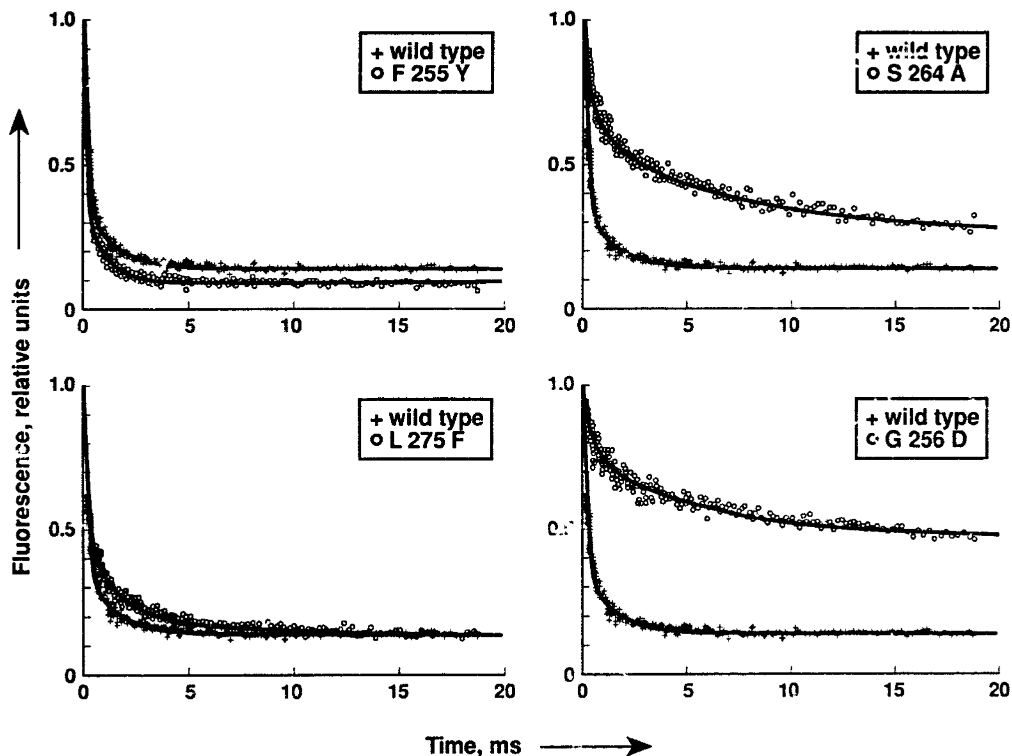

Fig. 1. Chlorophyll *a* fluorescence decay after the last of the three flashes in the wild type and D1 mutants of *C. reinhardtii*. Drawn lines are fits for the data (see Table 1 for values of constants and other parameters).

TABLE 1

Kinetic parameters for C. reinhardtii fluorescence yield decay after the third flash, $\tau_{slow} \geq 1$ *s in all cases*

Samples	A_{fast}	A_{int}	A_{slow}	τ_{fast} (μs) $\pm 50\ \mu$s	τ_{int} (μs) $\pm 200\ \mu$s	Slow/fast ratio $\frac{A_{slow}}{1 - A_{slow}}$
Fluorescence						
Wild type	0.63	0.22	0.15	260	1520	0.18
L275F	0.62	0.24	0.15	390	3480	0.17
V219I	0.51	0.32	0.16	270	2060	0.19
F255Y	0.72	0.18	0.10	260	1450	0.11
S264A	0.29	0.35	0.36	560	4770	0.64
G256D	0.24	0.28	0.47	430	5960	0.90
(Q_A)						
Wild type	0.66	0.21	0.13	320	2070	0.16
L275F	0.66	0.22	0.12	530	5930	0.16
V219I	0.55	0.32	0.13	240	2580	0.18
F255Y	0.77	0.14	0.09	360	2330	0.10
S264A	0.32	0.29	0.39	800	10390	0.60
G256D	0.27	0.30	0.43	560	11090	0.80

5-times than those of the wild type. The larger τ_{fast} of S264A than that of the wild type is in agreement with the conclusion of Etienne et al. [15]. We show that G256D also had larger τ_{fast}.

Etienne et al. [15] had suggested that in several D1 mutants (S264A/F255L; S264A; A255V/F211S) of *Synechocystis* 6714, the high fluorescence yield in the ms range, after the flash, was due to a decrease in the apparent equilibrium constant. In S264A of *C. reinhardtii*, however, high fluorescence yield in the ms range after the flash was suggested to be due mainly to an increased lifetime of the forward reaction, not to a change in apparent equilibrium constant. Results, discussed above, suggest that even the apparent equilibrium constant may also be altered in S264A.

Our results, thus far, suggest that in both S264A and G256D mutants of *C. reinhardtii*, in contrast to other mutants (L275F, V219I and F255Y), the fast fluorescence decay lifetime, as well as the apparant equilibrium of $Q_A^- Q_B \rightleftharpoons Q_A Q_B^-$ are several times smaller. A smaller apparent equilibrium constant was also suggested to occur in *Synechococcus* PCC 7942 D1 mutants through measurements of thermoluminescence bands due to $S_2Q_B^-$ and $S_2Q_A^-$ recombination (Gleiter et al. [26] and Ohad et al. [27]). The hierarchy of this change (largest to the smallest) was: D1-S264G > D1-F255Y/S264A ~ F255L/S264A ~ D1 S264A > F255Y ~ WT [27]. Furthermore, Taoka and Crofts [28], through an analysis of the pH-dependence of the Q_AQ_B reactions in thylakoids of a triazine-resistant S264 *Amaranthus hybridus* mutant, also showed a lowering of the apparent equilibrium constant. It is expected that similar measurements and analyses of thylakoids of *Chlamydomonas reinhardtii* mutants would yield further detailed information on differences among the mutants studied here.

It is generally accepted (see e.g. Ref. 15) that the slow (seconds' range) component of Chl *a* fluorescence yield decay reflects $S_2 Q_A^-$ to S_1Q_A reaction as it is also observed in DCMU-treated samples (see, e.g., Ref. 3), and that a portion of this component is suggested to belong to inactive PSII centers [23,24]. However, it is not possible to know, without additional measurements, what proportion of the slow component is due to the inactive centers, particularly in the mutants. The ratio of the slow to the fast PSII centers (± 0.1), when the total number of centers is normalized to 1, was quite high (0.6 to 0.9) in the S264A and G256D mutants as compared to the other three mutants and the wild type (0.11 to 0.19). This pattern also parallels that which is obtained from a comparison of the ratio (± 0.2) of the 'OI' phase (reflecting the centers that are slow in transferring electrons to Q_B, see, e.g., Ref. 16) and the 'IP' phase (reflecting the fast centers) of fluorescence transients [8]. Thus, G256D and S264A seem to have the largest fraction of slow PSII centers among the five mutants examined, and this may be one of the reasons for high $[Q_A^-]$ at longer times after the flash.

Van Rensen and Spätjens [29] showed that a *Chenopodium album* mutant, resistant to the herbicide triazine, had a higher proportion of the so-called 'β' centers [30]. Since the β centers overlap with the inactive non-Q_B PSII centers [30] and the triazine resistant *C. album* mutant is a D1-S264G mutant, our results on G256D and S264A mutants may suggest a possibility that even a single amino acid mutation may cause structural changes, leading to an increase in the fraction of 'slow' centers. Such phenomena are not surprising in view of the results on mutants of photosynthetic bacteria (see a review by Deisenhofer and Michel [31]). Furthermore, Etienne et al. [15] showed that, in contrast to the wild type, S264A mutants

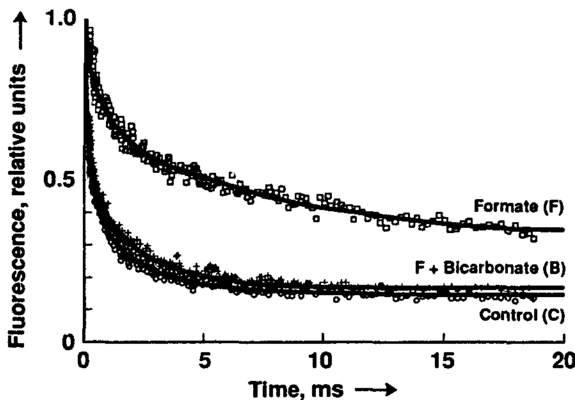

Fig. 2. Fluorescence decay curves after the last of the three flashes in D1-V219I mutant in control (C), with 200 mM formate (F) and formate-treated samples restored with 20 mM bicarbonate (F + B).

(DCMU IIA and IIB) of *Synechocystis* 6714, that had a high (36–38%) amplitude of slow fluorescence decay component, had a 1.5-times higher damping of oxygen oscillations and a 7–8°C shift towards lower temperature of the 'B' band of thermoluminescence. Similar results were found for the S264A mutant of *C. reinhardtii*. However, their wild type control samples also had high damping of oxygen oscillations. Further experiments on the O_2 evolution and thermoluminescence are necessary, on the mutants used here, in order to reach firm and detailed conclusions.

Bicarbonate-reversible formate effect

A 20 mM bicarbonate-reversible 100 (or 200) mM formate effect on Chl *a* fluorescence decay was observed in the wild type and the mutants tested. Fig. 2 shows an example of the phenomenon in the V219I mutant. Analysis of $[Q_A^-]$ data (Table II) showed that the lifetime of the fast phase (τ_{fast}) increased 8-times in S264A followed by 3-times in V219I and 1.1-times in L275F. These data are qualitatively comparable to the differential sensitivity of the biocarbonate-reversible formate effect on the Chl *a* fluorescence transient in the same D1 mutants [8]. The effect on the amplitude of the fast component was, however, much less (only 2-fold) and less discriminatory among mutants. Qualitatively, all the results confirm the importance of L275 and S264 in the bicarbonate-reversible formate effect [8], but, unfortunately, provide no further clues as to the nature of the effect.

TABLE II

Kinetic parameters for $[Q_A^-]$ decay after the third flash, in formate-treated C. reinhardtii. $\tau_{slow} \geq 1$ s in all cases

Samples	A_{fast}	A_{int}	A_{slow}	τ_{fast} (μs)	τ_{int} (μs)
1 Wildtype control	0.51	0.24	0.25	330	1790
+ 100 mM formate	0.36	0.24	0.40	470	6710
2 L275Y control	0.48	0.28	0.24	480	4150
+ 100 mM formate	0.27	0.27	0.46	510	5250
3 V219I control	0.36	0.38	0.26	270	2160
+ 200 mM formate	0.17	0.35	0.48	610	8580
4 F255Y control	0.64	0.19	0.17	350	1800
+ 100 mM formate	0.23	0.38	0.39	790	9820
5 S264A control	0.18	0.31	0.51	460	6910
+ 200 mM formate	0.09	0.35	0.56	3450	36990

Concluding remarks

It appears that D1-S264 plays important roles in the binding of PQ, at the Q_B site, in the Q_A^- to Q_B^- reactions, including the electron and the proton transfer to the PQ pool, and in the formate/bicarbonate effect (Refs. 7, 8, 15 and this paper). However, V219, F255 and L275 may be of marginal importance for the reactions at the Q_AQ_B complex, but the role of L275 for the formate/bicarbonate effect needs to be further understood. Although the G256D mutant gives similar results to the S264A mutant, the role of G256 needs to be further examined with additional mutagenesis, since the change from glycine to aspartic acid is a rather drastic one. Continued research on the D1 mutants of *C. reinhardtii* holds promise for the understanding of the Q_B binding pocket and the bicarbonate binding niche in the reaction center of PS II.

We suggest that the major effect of S264A and G256D mutations may be to alter the distribution pattern of the binding of plastoquinone (PQ) at the Q_B binding site. As a consequence, the proportion of PS II centers with bound PQ decreases and those with unbound (non-Q_B) PQ increases. In addition, the kinetic characteristics of PS II centers with bound PQ are altered. For a discussion of the distribution patterns related to conformational substates in protein function, see Frauenfelder et al. [32] and Govindjee et al. [33].

Acknowledgements

We thank Ann-Lise Etienne for various suggestions during the preparation of the current version of our paper. We are grateful to Beatrix Schwarz for growing all the mutants (gifts from Jean-David Rochaix) and the wild type of *C. reinhardtii*. We are thankful for a grant given by the University Research Board, UIUC, Urbana, IL, the Academy of Science in Geneva (G), the Swiss National Science Foundation (31-27799.89 to RJS), and the University of Geneva (G) for financial support. Typing of the final copy of this manuscript by Trish Bright is greatly appreciated. Thanks are also due to the Artist Service of the School of Life Sciences for illustrations.

References

1 Lavorel, J. and Etienne, A-L. (1977). Curr. Top. Photosynth. Res. 1, 203–268.

2 Van Gorkom, H.J. (1986) in Light Emission by Plants and Bacteria (Govindjee, Amesz, J. and Fork, D.C., eds.), pp. 267–289, Academic Press, Orlando, FL.

3 Eaton-Rye, J.J. and Govindjee (1988) Biochim. Biophys. Acta 935, 237–257.

4 Duysens, L.N.M. and Sweers, H.E. (1963) in Studies on Photosynthetic Bacteria and Microalgae (Japanese Society of Plant Physiologists, ed.), pp. 353–372, University of Tokyo Press, Tokyo.

5 Trebst, A. (1986) Z. Naturforsch. 41c, 240–245.

6 Diner, B.A., Petrouleas, V. and Wendoloski, J.J. (1991) Physiol. Plant 81, 423–436.

7 Erickson, J.M., Pfister, K., Rahire, M., Togasaki, R.K., Mets, L. and Rochaix, J-D. (1989) The Plant Cell 1, 361–371.

8 Govindjee, Schwarz, B., Rochaix, J-D. and Strasser, R.J. (1991) Photosynth. Res., 27, 199–208.

9 Pfister, K. and Arntzen, C.J. (1979) Z. Naturforsch. 34c, 996–1009.

10 Bowes, J., Crofts, A.R. and Arntzen, C.J.A. (1980) Arch. Biochem. Biophys. 200, 303–308.

11 Vermaas, W.F.J. (1984) Ph.D. Thesis, University of Wageningen, The Netherlands.

12 Demeter, S., Vass, I., Hideg, E. and Sallai, A. (1985) Biochim. Biophys. Acta 806, 16–24.

13 Ort, D., Ahrens, W.H., Martin, B. and Stoller, E.W. (1983) Plant Physiol. 72, 925–930.

14 Janssen, M.A.K. and Pfister, K. (1989) Z. Naturforsch. 45c, 441–445.

15 Etienne, A-L., Ducruet, J-M., Ajlani, G. and Vernotte, C. (1990) Biochim. Biophys. Acta 1015, 435–440.

16 Cao, J. and Govindjee (1990) Biochim. Biophys. Acta 1015, 180–188.

17 Gorman, D.S. and Levine, R.P. (1965) Proc. Natl. Acad. Sci. USA. 54, 1665–1669.

18 Schreiber, U. (1986) Photosynth. Res. 9, 261–272.

19 Xu, C., Rogers, S.M.D., Goldstein, C., Widholm, J.M. and Govindjee (1989) Photosynth. Res. 21, 93–106.

20 Leatherbarrow, R.L. (1987) ENZFITTER - a non-linear regression data analysis program for the IBM PC. A manual, Elsevier, Amsterdam.

21 Joliot, A. and Joliot, P. (1964). C.R. Acad. Sci. Paris 258, 4622–4625.

22 Chylla, R. and Whitmarsh, J. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), Vol. I, pp. 383–386, Kluwer Academic, Dordrecht.

23 Chylla, R.A. and Whitmarsh, J. (1989) Plant Physiol. 90, 765–772.

24 Crofts, A.R. and Wraight, C. (1983) Biochim. Biophys. Acta 726, 148–185.

25 Robinson, H. and Crofts, A.R. (1987) FEBS Lett. 153, 221–226.

26 Gleiter, H.M., Ohad, N., Hirschberg, J., Fromme, R., Renger, G., Koike, H. and Inoue, Y. (1989) Z. Naturforschg. 45c, 353–358.

27 Ohad, N., Amir-Shapira, D., Koike, H., Inoue, T., Ohad, I. and Hirschberg, J. (1989) Z. Naturforschg. 45c 402–408.

28 Taoka, S. and Crofts, A.R. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), Vol. I, pp. 547–550, Kluwer Academic, Dordrecht.

29 Van Rensen, J.J.S. and Spatjens, E.E.M. (1986) Z. Naturforschg. 42c, 794–797.

30 Melis, A. (1991) Biochim. Biophys. Acta 1058, 87–106.

31 Deisenhofer, J. and Michel, H. (1991) Annu. Rev. Biophys. Biophys. Chem. 20, 247–266.

32 Frauenfelder, H., Parak, F. and Young, R.D. (1988) Annu. Rev. Biophys. Biophys. Chem. 17, 451–479.

33 Govindjee, Van de Ven, M., Preston, C., Seibert, M. and Gratton, E. (1990) Biochim. Biophys. Acta 1015, 173–179.