Biochimica et Biophysica Acta, 680 (1982) 258–264
Elsevier Biomedical Press

BBA 41082

THE DEACTIVATION OF PHOTOSYSTEM II IN *CHLORELLA* AS A SECOND-ORDER PROCESS

JOHN SINCLAIR and CATHERINE COUSINEAU

Department of Biology, Carleton University, Ottawa, Ontario, K1S 5B6 (Canada)

(Received November 30th, 1981)

Key words: Photosystem II deactivation; Oxygen evolution; Electron transport; (Chlorella)

The kinetics of deactivation of the S_3 state in *Chlorella* have been observed under a variety of conditions. The S_3 state appears to decline in a dark period coming after a sequence of 30 saturating flashes in a second-order reaction, the rate constant of which is $0.132/[S_3^*]\ s^{-1}$ and which involves an electron donor, D_1, of concentration $1.25[S_3^*]$ where $[S_3^*]$ is the concentration of the S_3 state when the oxygen yield of the light flashes is constant. If a 1 min period of 650 nm illumination is employed after the sequence of flashes, the subsequent S_3 state deactivation kinetics are more complex. There is an initial phase of S_3 state deactivation, accounting for about 35% of the original S_3 state, which is complete in less than 100 ms. The remaining 65% of the S_3 state appears to deactivate in a second-order reaction, the rate constant of which is $1.36/[S_3^*]\ s^{-1}$ and which involves an electron donor of initial concentration $0.58[S_3^*]$. If a 1 min period of 710 nm illumination comes after the 30 flashes, at least 98% of the S_3 state deactivates according to first-order kinetics. It is shown that this can be explained using a second-order model if there is an electron donor present of which the concentration is large compared with $[S_3^*]$. However, S_3 state deactivation observed after 5 min of dark and two saturating flashes can be described neither by a first-order model nor a second-order model. Deactivation of the S_2 state after a 5 min dark period and one saturating flash follows second-order kinetics with a rate constant of $0.2/[S_3^*]\ s^{-1}$ and appears to involve an electron donor of initial concentration $1.3[S_3^*]$. Arguments are presented which tend to rule out the primary electron acceptor to Photosystem II as being any of the electron donors but it appears quite possible that the large plastoquinone pool is involved.

Introduction

When dark-adapted *Chlorella* or isolated chloroplasts are exposed to a suitable sequence of brief saturating light flashes, the resulting oxygen yields exhibit a damped oscillation of periodicity four. A study of this phenomenon led Kok and co-workers [1,2] to propose the existence of a complex for storing positive charge which is located in the electron-transport chain between the water-splitting enzyme system and the PS II reaction centre, *P*-680. According to this proposal, the complex acquires positive charges in light-driven steps until it has received a total of four when it enters a chemical reaction which releases oxygen and returns the complex to its zero-charge state. It has been found that complexes storing either two or three positive charges are unstable in the dark and disappear in a process called deactivation. This was studied in *Chlorella* by Joliot et al. [3] who showed that the S_3 state (i.e., a complex with three positive charges) disappeared in the dark in a

Abbreviations: PS, photosystem; Tricine, *N*-tris-(hydroxymethyl)methylglycine; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

005-2728/82/000–0000/$02.75

process which had a half-time of about 4.8 s for the first 20 s (approximately) but then proceeded much more slowly. Deactivation was studied in isolated chloroplasts by Forbush et al. [2] who found it necessary to interpret their results using a model which combined first- and second-order kinetics. In this paper, we have studied the deactivation of the S_3 state in *Chlorella* and attempted to analyse the results in terms of a simple second-order model evaluating, where possible, the rate constants and the concentration of the electron donor involved.

Methods

The organism used in this study was *Chlorella vulgaris*, obtained from Carolina Biological Supply Co., and cultivated as described by Sinclair and Arnason [4]. They were observed in an oxygen polarograph where they were immersed in a medium containing 100 mM NaCl, 50 mM Tricine, pH 7.6. As described below, the cells, which were deposited as a monolayer on the platinum electrode, were subjected to sequences of brief saturating light flashes. Details of the flash-generation apparatus and methods of measuring the relative oxygen yields of the flashes can be obtained from Ref. 5. All experiments were done at 23 (±1)°C.

Several different procedures were used when studying S_3 state deactivation which are designated as methods A, B and C.

Method A. This involved essentially the same light protocol as that used by Bouges-Bocquet et al. [6], i.e., the monolayer of cells was left for 5 min in darkness, given two light flashes separated by 0.3 s which was followed by a variable dark period and then a sequence of 30 light flashes (separation 0.3 s). The O_2 yield of the flash terminating the variable dark period was taken as proportional to the amount of S_3 state remaining after the dark period. The O_2 yields were normalized with respect to the O_2 yield of a flash given 0.3 s after the second flash.

Method B. The cells were exposed to sequences of 30 flashes (separation 0.3 s) between which intervened a dark period of variable duration. The oxygen yield of the first flash in each sequence was taken as a measure of the S_3 state surviving the dark period. The oxygen yield was normalized with respect to the mean oxygen yield of the last five flashes in the sequence

Method C. The cells were illuminated for 1 min by a light beam which had passed through either a 650 nm or a 710 nm interference filter. This constituted a preillumination period and was succeeded by a variable length dark period and then a sequence of 30 brief light flashes. The oxygen yield of the first flash was taken as a measure of the S_3 state and it was normalized with respect to the oxygen yield of a light flash given 2 s prior to the end of preillumination. In these experiments, it was also possible to measure the apparent rate of oxygen evolution during the preillumination period by observing the direct current flowing in the measuring circuit.

Prior to each 650 nm illumination, a 5 min dark period was included in the experimental procedure. It was found that without the 5 min dark period, repeated identical experiments gave progressively different results whereas with the 5 min dark period intervening, repeated experiments gave results which were in close agreement. This 5 min dark period was not necessary with the 710 nm experiments which gave highly reproducible results.

When we fit a second-order model to our results we assume that the S_3 state interacts with an electron donor D as follows: $S_3 + D \rightarrow S_2 + D_{ox}$, where D_{ox} is the oxidized form of D.

If $[S_3]_0$ and $[D]_0$ are the concentrations at the start of deactivation then it follows that:

$$[S_3]_0 - [S] = [D]_0 - [D] \tag{1}$$

at any time during this process.

The time course of the reactions is described by

$$\frac{d[S_3]}{dt} = -k[D][S_3] \tag{2}$$

where k is the rate constant. The solution is:

$$\ln\frac{[S_3]}{[S_3]_0} = \ln\frac{[D]}{[D]_0} + k([S_3]_0 - [D]_0)t \tag{3}$$

Using Eqn. 1 to eliminate [D] from Eqn. 3 permits us to fit the second-order model to our results

by choosing a value of $[S_3]_0/[D]_0$ which yields a straight line for the plot of $\ln[S_3]$ vs. time. When this is achieved it is possible to evaluate both $[D]_0$ and k in terms of $[S_3]_0$. To make the results of different experiments comparable we have in fact expressed $[D]_0$ and k in terms of $[S_3^*]$, the concentration of the S_3 state when the flash yield has reached a steady value.

Results

The kinetics of S_3 state deactivation obtained with method A are illustrated by trace A in Fig. 1. Since over 98% of the S_3 state disappeared during the course of this experiment, we considered only values of $[S_3]_0/[D]_0 \leq 1$ when attempting to fit a second-order model. Traces B and C have been drawn through the points computed for $[S_3]_0/[D]_0$ equal to 0.9 and 0.7, respectively. It is quite apparent that neither of these lines is even approximately straight which was true of all the values tried between $[S_3]_0/[D]_0$ equal to 1 and 0.1. For small values of this ratio a second-order model yields first-order kinetics.

When method B was used to observe S_3 state deactivation the results described by trace A in Fig. 2 were obtained. When $[S_3]_0/[D]_0$ was assigned a value of 0.8 the points through which trace B has been drawn were the result of fitting the second-order model. There is an excellent fit obtained in this case between experiment and theory. The half-time associated with trace B is 20.5 s which yields a value of $0.132/[S_3^*]\ s^{-1}$ while $[D]_0 = 1.25[S_3^*]$.

The kinetics of deactivation observed with method C depended on the wavelength of illumination as shown in Fig. 3 which was obtained using a single monolayer of *Chlorella*. The intensities of 710 and 650 nm preillumination were chosen so as to equalize the apparent rates of oxygen evolution. The steady-state flash yields were the same in the two cases. The results indicate that after 710 nm preillumination (open circles), at least 98% of the S_3 state originally present decays according to first-order kinetics. The slope of the best-fitting straight line corresponds to a half-time of 4.3 s and intercepts the ordinate at 0.96 relative units. The results following 650 nm illumination (solid circles) are obviously more complex and to

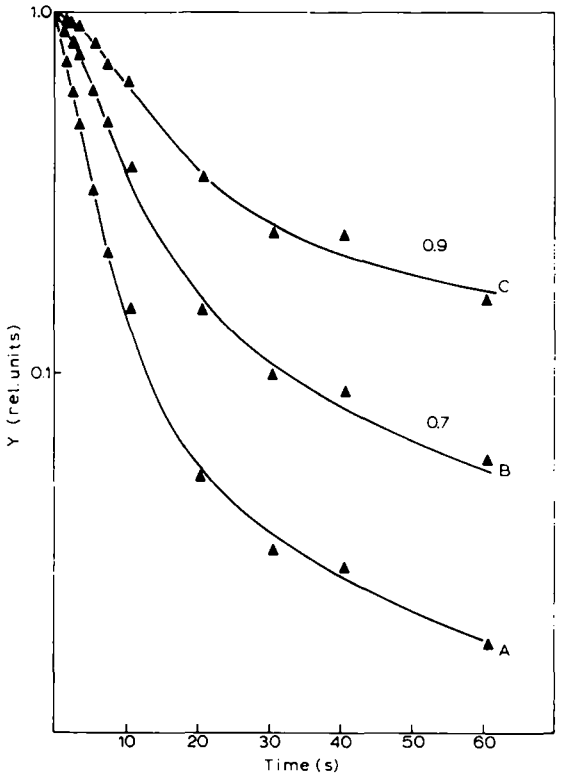

Fig. 1. The deactivation of the S_3 state was observed using method A (see Methods) for a monolayer of *Chlorella* cells. Trace A is drawn through points for which Y is the relative flash yield of oxygen measured after a given period of darkness. The oxygen yields were normalised against the oxygen yield of a flash given after 0.3 s of darkness. To test whether the experimental results could be described by a second-order model, Eqns. 1 and 3 (see Methods) were used and a value of $[S_3]_0/[D]_0$ was assumed. This made it possible to calculate values of $[D]/[D]_0$ for each period of darkness and these were multiplied by the corresponding experimental results to yield the points described by traces B and C for which $[S_3]_0/[D]_0$ was 0.7 and 0.9, respectively. If the second-order model fits the experimental results these computed points should be on a straight line as indicated by Eqn. 3.

assist in their analysis we have drawn the best-fitting straight line through the results obtained during the first 5 s of darkness. The half-time of this line is 2.1 s and it intercepts the ordinate at 0.65 relative units. The low value of this intercept suggested to us that there might be an initial phase of S_3 state deactivation which proceeded very rapidly. This suggestion was investigated in the experiment illustrated in Fig. 4 which was performed on another monolayer of *Chlorella* under

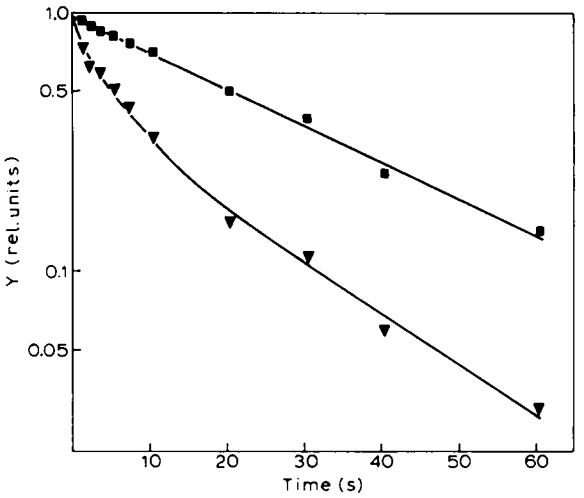

Fig. 2. The deactivation of the S_3 state was observed using method B for a monolayer of *Chlorella* cells. *Y* for the lower points is the relative oxygen flash yield at a given dark time, the yield having been normalised with respect to the steady-state flash yield. The upper set of points represents an attempt to describe the experimental results with a second-order model for which $[S_3]_0/[D]_0 = 0.8$ using the procedure described in the legend to Fig. 1.

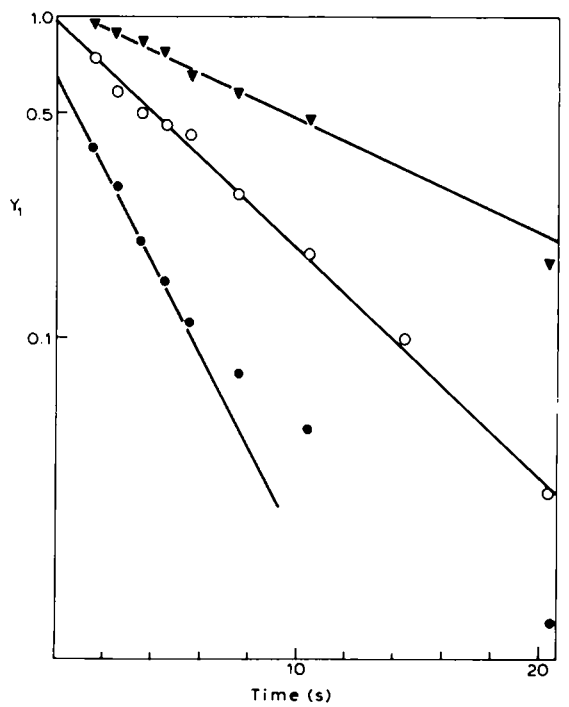

Fig. 3. The deactivation of the S_3 state for a monolayer of *Chlorella* observed with method C for a 650 nm preillumination (●) and 710 nm preillumination (○). The two intensities gave

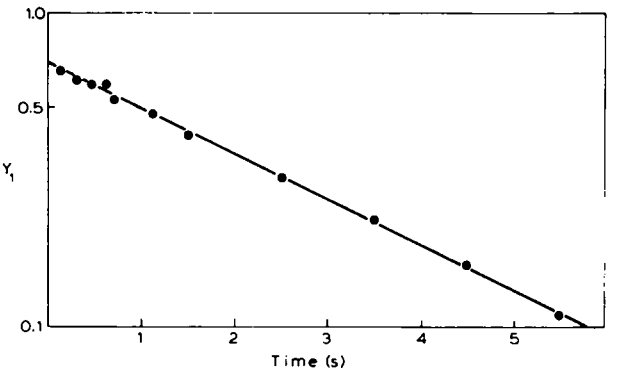

Fig. 4. S_3 state deactivation was observed for a monolayer of *Chlorella* using method C and the same intensity of 650 nm light used for Fig. 3. *Y* is the oxygen flash yield normalised with respect to the oxygen yield of a flash given 2 s prior to the end of illumination. Observations were made down to 100 ms of darkness and the best-fitting straight line calculated by regression analysis is drawn through the results.

identical conditions. A single exponential of half-time 2.1 s and intercepting the ordinate at 0.68 relative units gives a good description of the results between 100 ms and 5.5 s. This experiment confirms that a substantial fraction of S_3 state deactivation occurs very rapidly. The remaining 65% of S_3 state in Fig. 3 disappears much more slowly and we found that it was possible to describe this using a second-order model. This is shown by the triangular symbols in Fig. 3 which were calculated for $[S_3]_0/[D]_0 = 0.9$. Taking into account that (a) the half-time of the line through these points is 8.9 s, (b) only 65% of the original S_3 state is involved, and (c) $[S_3]_0$ was only 80% of $[S_3^*]$, it can be computed that $k = 1.36/[S_3^*]$ s^{-1} and $[D]_0 = 0.58[S_3^*]$.

The effect of varying the light intensity of both 710 and 650 nm light on the S_3 state deactivation kinetics was examined. Below about 1000 erg/cm^2

equal apparent photosynthetic rates. *Y* represents the oxygen flash yield normalised with respect to the oxygen yield of a flash given 2 s prior to the end of the preillumination. The line through the 710 nm results represents the best-fitting straight line obtained by regression analysis while the line through the 650 nm results in a similar line for the first 5 s of darkness. The intercept of the latter line on the *Y*-axis is 0.65. The model-fitting procedure used in Fig. 1 was applied to the 650 nm results now normalised with respect to 0.65 and setting $[S_3]_0/[D]_0 = 0.9$. (▼) Results of the latter calculation.

per s of 710 nm light or about 200 erg/cm^2 per s of 650 nm light, the half-times of deactivation became significantly larger whereas above these light intensities there was very little dependence on intensity. The intensities used in the experiment shown in Fig. 3 were both in the region where there is little sensitivity to intensity.

The addition of 10^{-7} M DCMU to the bathing medium caused an 80% inhibition of the steady-state flash yield. In a continuation of the experiment shown in Fig. 3 deactivation was observed in the presence of the inhibitor following the same two types of preillumination. The two sets of results are virtually coincident (Fig. 5) and the best-fitting straight lines for the first 7.5 s of darkness have slopes corresponding to half-times of 3.3 s (710 nm) and 3.2 s (650 nm). The value of the intercepts are 1.03 relative units for 710 nm and 0.94 relative units for 650 nm. Thus, the addition of DCMU appears to abolish the differences in deactivation due to preillumination and also the initial very fast phase of S_3 state deactivation seen after 650 nm illumination with uninhibited cells.

The deactivation of the S_2 state was also examined to see if this could also be described by a second-order model. A monolayer of cells was dark adapted for 5 min, given a single flash followed by a variable dark period which was terminated by a sequence of 30 flashes spaced at 0.3 s. The yield of the third flash was taken as a measure of the amount of S_2 state surviving the variable dark period (according to the method of Bouges-Bocquet et al. [6]). The deactivation of the S_2 state is shown in Fig. 6A along with our attempt to fit the kinetics to a second-order model (Fig. 6B). We have used an alternative solution to Eqn. 1 which is valid only when the concentration of the electron donor is equal to that of the S state at the start of deactivation. This solution is:

$$\frac{1}{[S_2]} = \frac{1}{[S_2]_0} + kt \qquad (3)$$

where $[S_2]_0$ is the concentration of S_2 state at the start of deactivation and k the rate constant. As shown in Fig. 6B, the results of $1/[S_2]$ plotted vs.

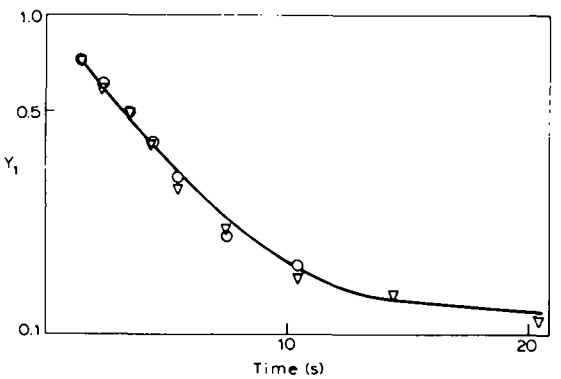

Fig. 5. S_3 state deactivation in the presence of 10^{-7} M DCMU was observed for the same monolayer of *Chlorella* used in Fig. 3. Method C was employed and also the same intensities of 650 nm (○) and 710 nm (▽) lights.

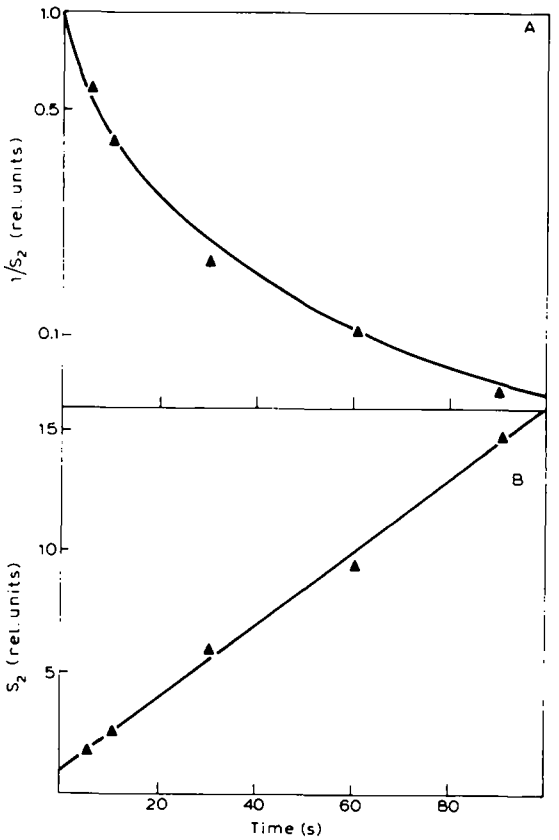

Fig. 6. (A) The deactivation of the S_2 state in a monolayer of *Chlorella* cells observed using the method described in Results. In B an attempt has been made to describe the experimental results by a second-order model for which $[D]_0 = [S_2]_0$. In such a situation, $1/[S_2]$ should increase linearly with time and intercept the ordinate at unity. In this figure, $1/[S_2]$ has been plotted against time and a straight line drawn through the points which intercepts the ordinate at unity.

time do yield a straight line which extrapolates through unity as required (the results were normalized with respect to $[S_2]_0$). The rate constant given by the slope of the line is $0.2/[S_3^*]\ s^{-1}$ and $[D] = 1.3[S_3^*]$.

Discussion

It has been demonstrated in this study that a second-order model can give a good description of the S_3 state deactivation kinetics observed with method B, with method C using 650 nm illumination (for the slow phase), and also for S_2 state deactivation. As discussed below, a second-order model can also describe (at least qualititatively) the S_3 state deactivation kinetics observed with method C after 710 nm illumination. While little can be said about the fast phase observed with method C after 650 nm illumination, it is clear that the results obtained with method A do not conform to a second-order model. Since method A included a 5 min dark period which was absent in method B, it may be that this has an important influence in creating the observed kinetics. Kok and Velthuys [7] observed that the combined oxygen yields of the first four flashes of light given to a sample of *Chlorella* kept in darkness decrease as the period of darkness is increased. The combined yield after 5 min of darkness was only 65% of that found after only a few seconds of darkness. The authors claimed that a more reducing environment developed within the cells in the dark so that Q, the primary electron acceptor to PS II, and also the other redox pools between the two photosystems became more reduced. In the present case, such a development could well increase the number of electron donors available for S_3 state deactivation and hence the kinetics would not be explicable in terms of a single second-order reaction. If this explanation is correct, it then becomes surprising that the method used to observe S_2 state deactivation, which also included a 5 min dark period, yielded results which are well described by a second-order model. Perhaps this difference can be resolved if the S_2 state is less accessible to these extra electron donors.

The analysis of our results (in terms of a second-order model) suggests the existence of several electron donors for S_3 state deactivation. The results obtained form method B suggest a donor D_1 of initial concentration $1.25[S_3^*]$ which takes part in a reaction, the rate constant of which is $0.132/[S_3^*]\ s^{-1}$. These values are very similar to those obtained for S_2 state deactivation which were $1.3[S_3^*]$ and $0.2[S_3^*]\ s^{-1}$ and raises the possibility that the same electron donor reacting with an identical or very similar chemical group is involved in the two deactivation reactions. Although no detailed information is available about the very fast phase of deactivation after 650 nm illumination, the very fast kinetics and the abolition of this phase in the presence of DCMU strongly suggest that there is another electron donor, D_2, involved in this reaction. The slow phase of S_3 state deactivation after 650 nm illumination seems to involve a donor, D_3, of initial concentration $0.58[S_3^*]$ and having a rate constant equal to $1.37/[S_3^*]\ s^{-1}$. As shown in Fig. 3, the S_3 state observed with method C after 710 nm illumination deactivates almost completely according to first-order kinetics. This is explicable on the basis of a second-order model if the initial concentration of the electron donor involved is much greater than $[S_3]_0$. Incorporation of this assumption into the second-order model yields a first-order version of the solution in Eqn. 3, namely:

$$\ln \frac{[S_3]}{[S_3]_0} = -k[D]_0 t \qquad (4)$$

As a consequence, we postulate the existence of a donor D_4 operative here when method C was used with 710 nm preillumination and of initial concentration much larger than $[S_3^*]$.

An earlier study by Lemasson [8] demonstrated that the kinetics of deactivation of the S_3 state in *Chlorella* proceed more slowly at lower light intensities. We have confirmed this finding in this study for both 650 nm (slow phase of deactivation) and 710 nm illumination. Referring to Eqns. 3 and 4, we would suggest that the intensity dependence of the deactivation kinetics could arise either from the rate constant being sensitive to light intensity or perhaps more plausibly the value of $[S]_0 - [D]_0$ in Eqn. 3 or $[D]_0$ in Eqn. 4 being light sensitive.

Lemasson and Barbieri [9] also studied the effect of the wavelength of preillumination on S_3 state deactivation and found that if the *Chlorella*

cells were in state 1, characterised by a high enhancement ratio (1.9), the half-time after 650 nm illumination was 1.6 s and after 710 nm illumination 4.8 s, whereas if the cells were in State 2, characterised by a low enhancement ratio (1.1), the half-time after either type of illumination was about 4 s. The results we obtained for the slow phase of 650 and 710 nm deactivation in Fig. 3 most closely resemble the results of Lemasson and Barbieri [9] for State 1 cells, however, measurements of the enhancement ratio for the cells used here gave values of about 1.1 corresponding to State 2. A second difference between our study and that of Lemasson and Barbieri [9] is that the latter workers did not observe the very fast phase of deactivation seen here after 650 nm illumination, nor did we ever see any sign of the transient acceleration of deactivation which these authors reported after 710 nm illumination.

The arguments presented above make it possible that there are four different electron donors D_1, D_2, D_3, D_4, which can take part in S_3 state deactivation under different conditions. While we cannot conclusively identify any of these donors, we will consider the likelihood that any of them is Q, the primary electron acceptor to PS II as suggested by Lemasson and Barbieri [9] and Bouges-Bocquet et al. [6]. In our experiments, we have observed a decline in the number of PS II centres which will respond to a single excitation by evolving a molecule of oxygen. Such a centre must have an oxidized Q molecule and a charge-storage complex bearing three positive charges and if either of these entities is reduced, it will contribute to the deactivation process. From this it follows that the direct reduction of the charge-storage complex by reduced Q does not influence the kinetics observed here, since neither before nor after the electron transfer would such a centre be able to evolve oxygen. Thus, none of the donors suggested by this study can be identical with Q.

As an alternative to Q, we suggest that D_4 could be the large pool of plastoquinone between the photosystems. This is primarily because D_4 appears to be present in such a large amount. However, D_4 is apparent only after 710 nm illumination when the action of non-cyclic electron transport would oxidize the plastoquinone pool. The work of Teichler-Zallen and Hoch [11] showed that cyclic electron transport was very rapid when far-red illumination was used with *Anacystis* and *Chlamydomonas*. If this were also true for *Chlorella*, plastoquinone could well be reduced after 710 nm illumination which would be in accord with our hypothesis. Our finding that D_4 is absent when DCMU is present could be explained by the action of non-cyclic electron transport from plastoquinone causing an oxidation of this substance. We would also like to suggest the possibility that D_2 is a reduced form of R [10]. D_2 is present after 650 nm illumination but not after 710 nm illumination as would be expected for R. Since R is thought to be the binding site for DCMU [12], its oxidation by the S_3 state could well be prevented by the inhibitor which would accord with the disappearance of D_2 in the presence of DCMU. A final point should be made about the phase of S_3 state deactivation in which D_2 participates. It is completed within a very brief time (100 ms) and it is possible that it represents a cyclic pathway around PS II which has functional significance in the light.

Acknowledgment

This research was funded by the Natural Sciences and Engineering Council of Canada.

References

1 Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475
2 Forbush, B., Kok, B. and McGloin, M. (1971) Photochem. Photobiol. 14, 307–321
3 Joliot, P., Joliot, A., Bouges, B. and Barbieri, G. (1970) Photochem. Photobiol. 14, 287–305
4 Sinclair, J. and Arnason, T. (1976) Biochim. Biophys. Acta 368, 393–400
5 Arnason, T. and Sinclair, J. (1976) Biochim. Biophys. Acta 449, 581–586
6 Bouges-Bocquet, B., Bennoun, P. and Taboury, J. (1973) Biochim. Biophys. Acta 325, 247–254
7 Kok, B. and Velthuys, B.R. (1976) in Research in Photobiology (Castellani, A., ed.), pp. 111–119, Plenum Press, New York
8 Lemasson, C. (1970) C.R. Acad. Sci. Paris Ser. D, 270, 350–353
9 Lemmason, C. and Barbieri, G. (1971) Biochim. Biophys. Acta 245, 386–397
10 Velthuys, B.R. and Amesz, J. (1974) Biochim. Biophys. Acta 333, 85–94
11 Teichler-Zallen, D. and Hoch, G. (1967) Arch. Biochem. Biophys. 120, 227–230
12 Pfister, K. and Arntzen, C.J. (1979) Z. Naturforsch. 34c, 996–1009