Biochimica et Biophysica Acta, 460 (1977) 247–258

BBA 47295

DEPENDENCE OF THE DEACTIVATION REACTIONS OF PHOTOSYSTEM II ON THE REDOX STATE OF PLASTOQUINONE POOL A VARIED UNDER ANAEROBIC CONDITIONS

EQUILIBRIA ON THE ACCEPTOR SIDE OF PHOTOSYSTEM II

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(Received September 30th, 1976)

SUMMARY

Dark adapted spinach chloroplasts and *Chlorella* with variably reduced plastoquinone pools were given 1 or 2 saturating flashes. Under these conditions, the rate of deactivation of state S_2 of the oxygen evolving site of Photosystem II (B. Kok, B. Forbush, M. McGloin (1970) Photochem. Photobiol. 11, 457–475) is highly dependent on the pool redox state, undergoing a nearly 10-fold acceleration upon transforming the plastoquinone pool (A) from 100 % oxidized to 90 % reduced. Deactivation of state S_3 is unaffected by the same variation of the pool redox state.

These observations are attributed to a high concentration of Photosystem II reduced primary electron acceptor, Q^-, coincident with the formation of S_2 and a low concentration coincident with the formation of S_3, under the conditions of highly reduced plastoquinone pool. Simultaneous determination of Q^- and A^{2-} result in an estimated equilibrium constant of 15–20 for reaction $Q^-B \rightleftarrows QB^-$ and a value greater than 50 for equilibrium $Q^-B^- \rightleftarrows QB^{2-}$, where B is the secondary electron acceptor described by B. Bouges-Bocquet ((1973) Biochim. Biophys. Acta. 314, 250–256) and B. R. Velthuys and J. Amesz ((1974) Biochim. Biophys. Acta. 333, 85–94). It is proposed that doubly reduced B becomes protonated in the last reaction.

INTRODUCTION

A number of authors [1–5] have studied the equilibria relating the redox state of primary acceptor Q (a plastoquinone) [6, 34] to that of the plastoquinone pool, A. Rather complicated results were obtained, indicating an equilibrium constant, high in the dark [3, 5] and low in the light [1–3]. In addition, there was evidence for a heterogeneity of the pool [7] containing two subpools of low and high equilibrium constants relative to Q [2, 4, 5].

That a heterogeneity existed became clearly established with the discovery of the intermediate acceptor, B, [8, 9] (a plastoquinone) [10, 11] located between Q and A. B accepts electrons one at a time from Q and upon accumulating an electron pair,

transfers its reducing equivalents to the A pool. Thus at least three equilibria relate Q to A. These are

$$B^{2-}+A \underset{K_1}{\rightleftarrows} B+A^{2-},\ Q^-B \underset{K_2}{\rightleftarrows} QB^-, \text{ and } Q^-B^- \underset{K_3}{\rightleftarrows} QB^{2-}.$$

K_1 has already been shown to be close to 1 [11]. An attempt will be made here to quantitatively determine K_2 and K_3. To do so, the redox states of Q, B, and A were measured simultaneously under atmospheres of varying oxygen content, which permitted a control over the redox state of the plastoquinone pool. This control results from the fact that in algal cells, reduction of the pool by an unknown endogenous reductant is offset by an oxidation by molecular oxygen [12]. In isolated spinach chloroplasts, lacking this reductant, the pool can nonetheless be reduced through illumination and subsequently reoxidized, during a given time, to an extent determined by the background oxygen concentration [11].

In a previous paper [11] it was shown that, where the pool was reduced by more than 90 %, practically all centers capable of producing oxygen in three flashes, were in state QB^{2-} just prior to the start of illumination. The sequence of events before and after the first and second saturating light flashes is shown below. S represents the states of the oxygen evolving site according to the model of Kok et al. [13].

$$S_1QB^{2-} \xrightarrow{h\nu} S_2Q^-B^{2-} \underset{K_1}{\overset{A^{2-}}{\rightleftharpoons}} S_2Q^-B \underset{K_2}{\rightleftharpoons} S_2QB^- \xrightarrow{h\nu} S_3Q^-B^- \underset{K_3}{\rightleftharpoons} S_3QB^{2-} \underset{K_1}{\overset{A^{2-}}{\rightleftharpoons}} S_3QB \quad (1)$$

Van Best and Duysens [14] later extended this analysis through fluorescence measurements, under anaerobic conditions and showed that all centers were in state Q^-B^{2-} after the first flash, and not just those centers which give oxygen after three flashes.

Lavorel [15] proposed that luminescence emission arises through a Photosystem II donor-acceptor side charge recombination, with Q^- as the reduced substrate. This proposal was strongly supported by the experiments of Bennoun [16] which showed that in the presence of DCMU, Q^- is oxidized exclusively by Z^{2+} with kinetics which correlate with the luminescence decay [17]. Finally, Joliot et al. [18] demonstrated that deactivation of states S_2 and S_3 was primarily responsible for luminescence emission in the absence of DCMU. Consequently, $-dS_2/dt$ and $-dS_3/dt$ should be proportional to the concentration of Q^-. Following single flash excitation of dark adapted anaerobic chloroplasts (Eqn. 1) the deactivation rate of S_2 should be a function of equilibrium constants, K_1 and K_2, and A^{2-}, while that for S_3 after double flash excitation should depend on K_1, K_3 and A^{2-}. In this paper, we will explore the dependence of the initial deactivation rates of S_2 and S_3 on the redox state of the A pool. These measurements, which permit a determination of the Q^- concentration as a function of A^{2-} lead to a determination of K_2 and a minimum value for K_3.

MATERIALS AND METHODS

Chlorella pyrenoidosa were grown on Knop medium containing Arnon's trace elements A_5 and B_6. Prior to use, cells were suspended in 0.1 M potassium phosphate, pH 7.0, containing 0.1 M KCl, at a chlorophyll concentration of about 300 μg/ml.

Chloroplasts were prepared from market spinach according to the method of Avron [19] and stored at −70 °C in 0.05 M Tris · HCl buffer pH 7.5, containing 0.01 M NaCl, 0.4 M sucrose and 5 % dimethylsulfoxide. When used for oxygen measurements, chloroplasts were diluted to a chlorophyll concentration of 300 μg/ml in the same medium, containing, in addition, 0.1 M KCl but without dimethylsulfoxide and without an artificial electron acceptor.

Oxygen was detected using a polarograph similar to that described earlier [12], which permitted equilibration of cells or chloroplasts with atmospheres of varying oxygen content. These consisted of gas mixtures of 10, 1675, 7000 and 210 000 ppm O_2 in N_2, humidified by passage through a water bubbler. The gas flow rate was 40 ml/m.

Flash illumination was provided by Xenon flash lamps (General Radio, Stroboslave, Model 1539-A) with a 2 μs width at half-height. All flashes used in the experiments, to be described, were saturating.

The oxidized plastoquinone pool size, in chloroplasts, was determined by measuring the fluorescence induction under the same conditions as used for S state deactivation measurements, i.e. same preillumination, electrode polarization and background oxygen concentration. The oxidized pool size was taken to be equivalent to the area bounded by the fluorescence induction curve and F_{max} (maximum fluorescence yield) [2]. For these measurements, continuous exciting light was filtered through one BG 38 (Schott) and two 4-96 (Corning) blue filters. The illumination intensity was of the order of 40 photons per s-center. A photomultiplier (Radiotechnique XP 1002), placed close to the polarograph, detected the emitted fluorescence through one 2-64 (Corning) and two Rubylith Ulano red blocking filters.

By comparison with fluorescence induction in the presence of DCMU (assumed to be roughly one equivalent) the bounded area for fluorescence induction in aerobic chloroplasts was found to be on the order of 20 equivalents in agreement with a similar measurement by Forbush and Kok [2].

The rate of charge recombination (deactivation of S_2, oxidation of Q^-) in the presence of 10^{-5} M DCMU was determined as described by Bennoun [16] by measuring fluorescence induction curves at variable times after a preillumination just sufficient to block all centers in the charge separated state. The relative amount of Q^- reoxidized during a given dark interval corresponds to the area bounded by the fluorescence induction curve and F_{max}, compared to that obtained without preillumination [16]. For a description of the apparatus used, see Bennoun [20].

All experiments were performed at approximately 23 °C.

RESULTS

Chlorella, placed under an anaerobic atmosphere of several tens of ppm O_2 (30 min) undergo a greater than 90 % reduction of the plastoquinone pool in the dark [11]. The average oxygen yield of the first five flashes (320 ms apart) under these conditions, is diminished 7-fold relative to that of aerobic cells.

The rate of S_2 deactivation was determined (see Joliot et al. [18] and Forbush et al. [21]) through single flash excitation (generating S_2) followed a variable dark time, t, later by a series of 10 flashes (each 320 ms apart). Oxygen detected on the 2nd flash of the series gives the relative concentration of S_2 remaining after time t. The rate

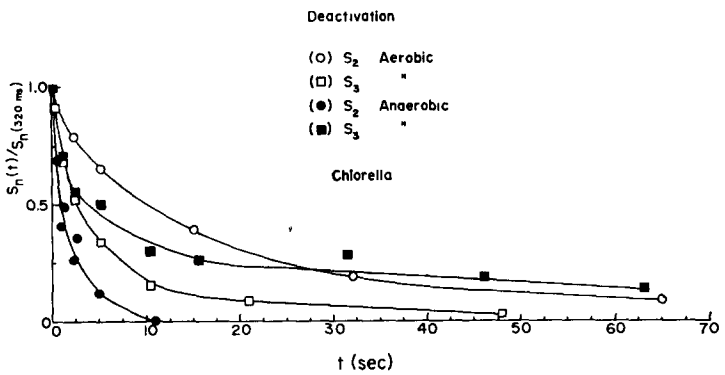

Fig. 1. Deactivation of states S_2 and S_3 in *Chlorella* under aerobic and anaerobic (pool > 90 % reduced) conditions. The rate of S_2 deactivation was determined through single flash excitation (generating S_2) of dark adapted cells, followed a variable dark time t, later by a series of 10 flashes, 320 ms apart. Oxygen detected on the second flash of the series gives the relative concentration of S_2 remaining after time t. The rate of S_3 deactivation was determined by giving 2 flashes (320 ms apart, generating S_3) follwed a variable time t, later by a series of 10 flashes, 320 ms apart. Oxygen detected on the first flash of the series gives the relative concentration of S_3 remaining after time t. In both series of experiments (S_2 and S_3) 5 min dark separated each measurement to allow dark readaptation. The curves are normalized relative to the oxygen yield at t = 320 ms.

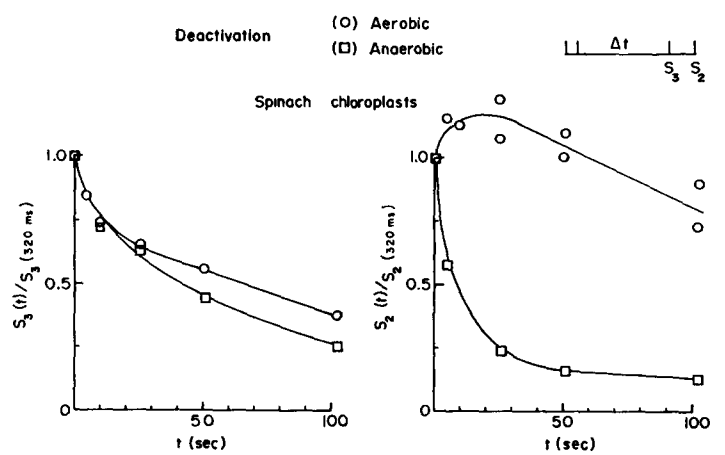

Fig. 2. Deactivation of states S_2 and S_3 in chloroplasts under aerobic and anaerobic (pool ≅ 70 % reduced) conditions. Anaerobic chloroplasts were given 2 flashes (320 ms apart, generating a mixture of S_2 and S_3) followed a variable dark time t, later by a series of 45 flashes (each 320 ms apart). Oxygen detected on the first flash of the series measures the relative concentration of S_3 remaining after time t. Oxygen detected on the second flash of the series measures S_2. 5 min dark readaptation separated each measurement. The aerobic experiment was performed in a similar manner except that the flash series was just sufficient to attain a steady state oxygen flash yield and 7 min dark separated each measurement.

of deactivation of S_3 was determined by giving 2 flashes (320 ms apart, generating S_3), followed a variable time, t, later by a series of 10 flashes (each 320 ms apart). Oxygen detected on the 1st flash of the series gives the relative concentration of S_3 remaining after time t. In both series of experiments (S_2 and S_3) 5 min dark separated each measurement to allow dark readaptation. The initial measurements were discarded until the algae, fully adapted to the background oxygen concentration and the flash program, gave reproducible data.

As shown in Fig. 1, S_2 deactivation, under these conditions, occurs with a $t_{\frac{1}{2}}$ of 0.8 s as opposed to 10 s under aerobic conditions. The deactivation times for S_3 appear to be little dependent on the background O_2 concentration (and the redox state of the plastoquinone pool) showing the same half-time decay of about 3 s. A slight divergence occurs at longer times, with, if anything, a slightly slower rate for anaerobic than aerobic conditions.

Similar behavior was observed, in chloroplasts (Fig. 2) for deactivation under aerobic and anaerobic conditions. A somewhat different experimental protocol was used from that of Fig. 1, permitting a simultaneous measurement of S_2 and S_3 deactivation.

Dark adapted chloroplasts equilibrated with several tens of ppm O_2 were given 2 flashes (320 ms apart) generating S_2 and S_3 followed a variable dark time, t, later by a series of 45 flashes (each 320 ms apart and sufficient to largely reduce the pool). Oxygen detected on the 1st flash of the series measures the relative concentration of S_3 remaining after time t. Oxygen detected on the 2nd flash of the series measures S_2 plus any S_3 missed on the preceeding flash (negligible). 5 min dark separated each measurement to allow complete relaxation to states S_0 and S_1. The initial measurements were discarded as for Fig. 1 until the data were reproducible. The pool was about 70 % reduced just before each measurement. The aerobic experiment was performed in a similar manner except that the flash series was just sufficient to obtain a steady state oxygen flash yield and 7 min dark separated each measurement.

As in the experiment of Fig. 1 (*Chlorella*) little difference is observed for the rate of deactivation of S_3 ($t_{\frac{1}{2}} \cong 50$ s, Fig. 2 left) under aerobic and anaerobic conditions. The kinetics for S_2 (Fig. 2, right) however, are quite different between the aerobic and anaerobic experiments. In the presence of 21 % O_2, S_3 decays to S_2 more rapidly than S_2 to S_1. Thus, after 2 flashes there is a transient increase of S_2 followed by a slow decay. With the pool largely reduced, no such increase occurs, not even a lag, indicating a decay of S_2 ($t_{\frac{1}{2}} < 10$ s) much accelerated relative to both that of S_3 and that for S_2 with the pool oxidized. Thus, aside from the fact that the decay times are slower for chloroplasts than for algae, the dependence of S_2 and lack of dependence of S_3 on the redox state of the plastoquinone pool are analogous to the algal experiments.

Considering the 5-fold difference in rate between S_3 deactivation (Fig. 2, left) and those kinetics measured in Fig. 2 right (anaerobic), it is unlikely that the fraction of centers in S_3 (most are in form S_3QB^{2-}) that did not undergo a photoreaction on the third flash and which are detected on the fourth, under anaerobic conditions, exceeds 20 %.

If it is assumed that Q^- is the principal reduced substrate for the deactivation of S_2 then a progressive reduction of the pool and the consequent increase in the concentration of Q^- should result in a regular acceleration in the deactivation rate of S_2 (Fig. 3). That this is in fact the case is shown below.

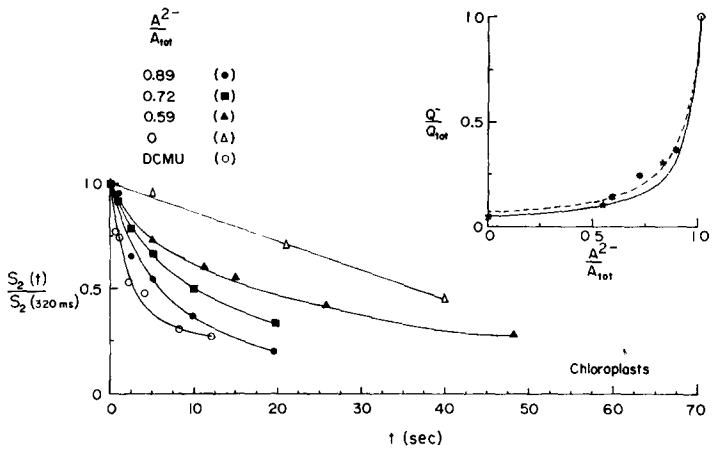

Fig. 3., left. Deactivation of state S_2 at different pool redox states, A^{2-}/A_{tot}, in chloroplasts adapted to atmospheres varying from several tens of ppm O_2 to air. The protocol of Fig. 1 was used except that each measurement was separated by 7 min dark readaptation and the flash series consisted of 19 flashes (each 320 ms apart). The deactivation of S_2 in the presence of 10^{-5} M DCMU was determined as described in Materials and Methods. The pool redox state, A^{2-}/A_{tot} was determined by a measurement of fluorescence induction (Materials and Methods, and Fig. 4), preceded by the same pretreatment as that for S_2 deactivation. Inset: comparison of the redox state of Q (Q^-/Q_{tot}) with that of the pool (A^{2-}/A_{tot}). The initial equilibrium Q^-/Q_{tot} was determined by comparison of the initial S_2 deactivation rate in 10^{-5} M DCMU ($Q^-/Q_{tot} = 1$) (○) with those measured at various oxidation states of the pool (e.g. Fig. 3, left). Two series of anaerobic experiments are shown (* and *) and an aerobic experiment (×). All experiments were performed on the same chloroplast preparation. Theoretical curves, calculated from Eqns. 4 and 5, are shown for $K_2 = 15$ (dashed line) and 20 (solid line).

Chloroplasts, in the absence of an artificial electron acceptor, were preadapted to various background oxygen concentrations. The protocol for the determination of the rate of S_2 deactivation was the same as that of Fig. 1, except that the flash series associated with each measurement consisted of 19 flashes (320 ms apart). Separating each measurement was a dark interval of 7 min, sufficient to allow complete relaxation of the S states to S_0 and S_1 and to reoxidize the pool to an extent determined by the background oxygen concentration. As in Figs. 1 and 2, the first few measurements were discarded until the data became reproducible.

The redox state of the pool was determined by a measurement of the fluorescence induction in continuous blue light (see Materials and Methods) preceded by the same pretreatment as for S_2 deactivation. Thus the S_2 deactivation rate and the redox state of the pool could be compared under the same conditions. Shown in Fig. 3 are the S_2 deactivation kinetics at the indicated pool redox states, A^{2-}/A_{tot} (A_{tot} = total concentration of plastoquinone, A^{2-} = concentration of reduced plastoquinone). A_{tot} is equivalent to the area bounded by F_{max} and the fluorescence induction curve under aerobic conditions (Fig. 4, lower right).

The concentration of Q^- was estimated by assuming that the initial rate of

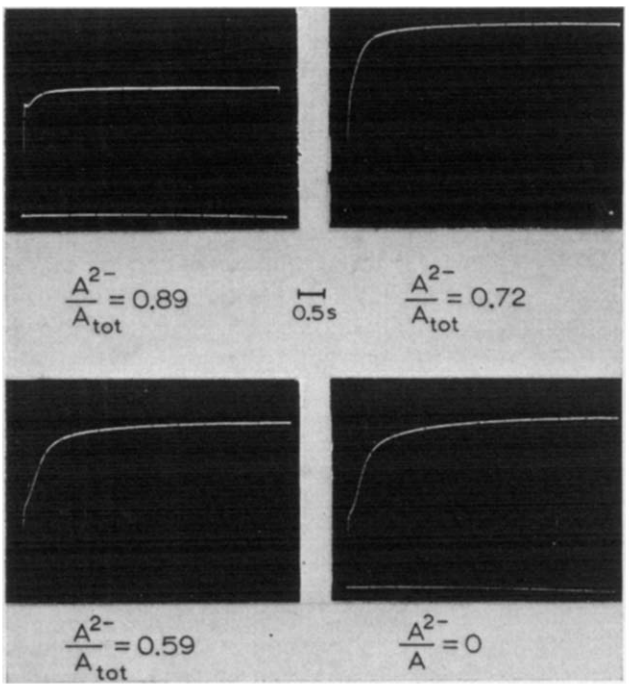

Fig. 4. Fluorescence induction curves for the S_2 deactivation experiments (*) shown in Fig. 3, left. The pretreatment was the same as for the deactivation measurements (i.e. same preillumination, dark time, and electrode polarization). The pool redox states were determined as described in Materials and Methods and are indicated below each curve. The base line, but not the vertical sensitivity, is the same for all four curves. For calculation of A^{2-}/A_{tot}, the curves were normalized to the same F_{max}.

deactivation was proportional to the initial Q^- concentration. The ratio Q^-/Q_{tot}* was taken to be equal to 1 for saturating light intensity in the presence of DCMU. The rate of S_2 deactivation in the presence of DCMU was assumed to be equal to the rate of Q^- reoxidation (detected by fluorescence) following preillumination [16]. The initial rates of the S_2 deactivation curves, in the absence of DCMU, and at various pool redox states, were then compared to that in the presence of DCMU. Only the initial rates were compared as the curves are obviously multiphasic (Fig. 3). The initial equilibrium Q^-/Q_{tot} is equivalent to the ratio:

$$\frac{\text{initial rate } (-\text{DCMU})}{\text{initial rate } (+\text{DCMU})}$$

Shown in Fig. 3 (inset) is Q^-/Q_{tot} as a function of the pool redox state A^{2-}/A_{tot} for two series of anaerobic experiments. The fluorescence induction curves for the series represented by * are shown in Fig. 4.

* Q_{tot}, maximum concentration of centers detectable in an experiment, i.e. all centers for fluorescence relaxation in the presence of DCMU, or those represented by the maximum yield on the oxygen detecting flash (3rd) of a deactivation experiment.

DISCUSSION

Deactivation of S_2

As shown in Eqn. 1 of the Introduction, the equilibrium concentration of Q^-, following single flash excitation of dark-adapted chloroplasts is a function of the pool redox state and the equilibrium constants, K_1 and K_2. Assuming that the initial rate of S_2 deactivation is proportional to Q^-, we can correlate Q^-/Q_{tot} with A^{2-}/A_{tot} determined by fluorescence induction. Turnover measurements [11], following a single flash, under anaerobic conditions, showed that the equilibrium constant $K_1 = (Q^-B)\,(A^{2-})/(Q^-B^{2-})\,(A)$ was close to 1. We thus have sufficient information to determine $K_2 = (QB^-)/(Q^-B)$.

Following the first flash, under anaerobic conditions, Photosystem II centers, detectable on the third flash, are in any one of three states as determined by anaerobic turnover measurements [11] and we can set their sum equal to 1. Thus,

$$QB_n^- + Q^-B_n + Q^-B_n^{2-} = 1 \tag{2}$$

(normalized to the maximum number of centers, Q_{tot}, detectable on the third flash). The concentration of QB^-, in the dark, preceding the first flash, is negligible under the anaerobic conditions used in these experiments (pool > 50 % reduced) and consequently there is no Q^-B^-, QB^{2-} or QB after the flash.

Under aerobic conditions, in the dark, QB^-, while less than QB, is not negligible ($\leqslant$ 30 % of the centers) and leads to a small error in the predicted Q^- concentration when the pool is fully oxidized.

Substituting Eqn. 2 in the equation for K_1

$$K_1 = \frac{(Q^-B_n)\,(A^{2-})}{(1-QB_n^- - Q^-B_n)\,(A)} \tag{3}$$

Substituting K_2 and solving for QB_n^-

$$QB_n^- = \left(\frac{(A^{2-})}{K_1K_2(A)} + \frac{1}{K_2} + 1\right)^{-1} = \left(\frac{1}{\left(K_1K_2\left(\frac{A_{tot}}{A^{2-}} - 1\right)\right)} + \frac{1}{K_2} + 1\right)^{-1} \tag{4}$$

where $A + A^{2-} = A_{tot}$
from Eqn. 2

$$\frac{Q^-}{Q_{tot}} = Q_n^- = 1 - QB_n^- \tag{5}$$

The theoretical dependence of Q^-/Q_{tot} on A^{2-}/A_{tot} for $K_1 = 1$ and $K_2 = 15$ (dashed line), 20(solid line) is plotted in Fig. 3. The experimental points are in reasonable agreement with the theoretical curves and a value of $K_2 \cong 15$.

There were a number of inherent simplifications in the foregoing analysis and we will now try to see how they affect the final result for K_2.

A^{2-}/A_{tot} was determined through comparison with the estimated aerobic pool size of 20 equivalents. A similar figure has been obtained previously by other authors measuring either fluorescence induction [2, 22] or the oxygen gush [23]. As pointed

out by Thorne and Boardman [22] and Radmer and Kok [4], the fluorescence estimate is subject to overestimation because of leakage to O_2 (Mehler reaction) [24]. Considering the leakage rate proposed by Radmer and Kok, this error probably amounts to 2–3 equivalents in the aerobic experiment performed here, but is non-existent in the anaerobic experiments.

No correction was made for Q as it represents only 1 equivalent under aerobic conditions and only half an equivalent (estimated from the Fo level, Fig. 4, upper left) under the most anaerobic conditions.

More serious, however, are any oxidizing equivalents located on the acceptor side of Photosystem I. Assuming the worst, that for point $A^{2-}/A_{tot} = 0.89$, the pool is in fact 100 % reduced, then the error due to their neglect is about 2 equivalents. It is unlikely that this number is much greater under aerobic conditions in that the Mehler, Q and two Photosystem I equivalents brings the pool size down to the 14–16 plastoquinone equivalents estimated spectroscopically by Stiehl and Witt [25].

If the maximum possible corrections are then made for both the overestimation of the oxidized pool size plus Photosystem I acceptor side equivalents, estimated above, then, with the exception of the point formerly at $A^{2-}/A_{tot} = 0.89$, all experimental points lie close to a theoretical curve for $K_2 = 20$.

Thus a more reasonable estimate for K_2 is somewhere between 15 and 20.

Based on a comparison of deactivation in the presence and absence of DCMU, Bouges-Bocquet [26] proposed that the equilibrium constant K_2 was $\cong 20$. Diner [11] mistakenly suggested that under anaerobic conditions the equilibrium constant for reaction $Q^-B^{2-} + A \rightleftarrows QB^- + A^{2-}$ might be close to 1 rather than the product $K_1 \cdot K_2$. The more complete analysis, reported here, shows instead that this equilibrium constant is $K_1 \cdot K_2 \cong 15$–20 and that K_2 is independent of the redox state of the pool. This result is in accord with the results of Schreiber and Vidaver [27] and the estimate of Van Best and Duysens [14] of a value of $K_1 \cdot K_2$ of at least 5, based on fluorescence measurements.

The equilibrium constant of 15–20 for reaction $Q^-B \rightleftarrows QB^-$ provides at least a partial explanation for the misses observed for charge storage on the donor and acceptor sides of Photosystem II. Any center whose acceptor side is in state Q^-B/QB^- (pool oxidized) will have approx. a ~ 6 % chance of not undergoing charge separation during a short saturating flash.

Deactivation of S_3

Unlike the deactivation of S_2, S_3 is, in the experiments reported here, practically independent of the redox state of the pool. For anaerobic chloroplasts, dark adapted for 7 min (pool reduced) all centers, detected in the deactivation experiments, are in state S_1QB^{2-} just prior to the first flash. After 2 flashes, those centers, detectable on the following flash are almost entirely in state S_3QB^{2-}. Under aerobic conditions (pool oxidized), 2 flashes produce essentially S_3QB and some S_3QB^-. These results imply that any Q^-, produced by reaction $QB^{2-} \rightleftarrows Q^-B^-$ is not normally the reduced substrate for S_3 deactivation, as reduction of B by the reduced pool (> 90 % of detectable centers in QB^{2-}, Fig. 1, and $\cong 70$ % for Fig. 2) would necessarily increase the concentration of Q^- even if the equilibrium constant, K_3, were high for reaction $Q^-B^- \rightleftarrows QB^{2-}$.

These results for S_3 differ from those reported by Lemasson and Barbieri [28]

4 Radmer, R. and Kok, B. (1973) Biochim. Biophys. Acta 314, 28–41
5 Velthuys, B. R. and Amesz, J. (1973) Biochim. Biophys. Acta 325, 126–137
6 Van Gorkom, H. J. (1974) Biochim. Biophys. Acta 347, 439–442
7 Joliot, P. (1965) Biochim. Biophys. Acta 102, 116–134
8 Bouges-Bocquet, B. (1973) Biochim. Biophys. Acta 314, 250–256
9 Velthuys, B. R. and Amesz, J. (1974) Biochim. Biophys. Acta 333, 85–94
10 Pulles, M. P. J., Van Gorkom, H. J. and Willemsen, J. G. (1976) Biochim. Biophys. Acta, 449, 536–540
11 Diner, B. (1975) in Proceedings of the IIIrd International Congress on Photosynthesis (Avron, M., ed.), pp. 589–601, Elsevier, Amsterdam
12 Diner, B. and Mauzerall, D. (1973) Biochim. Biophys. Acta 305, 329–352
13 Kok, B., Forbush, B. and McGloin, M. P. (1970) Photochem. Photobiol. 11, 457–475
14 Van Best, J. A. and Duysens, L. N. M. (1975) Biochim. Biophys. Acta 408, 154–163
15 Lavorel, J. (1969) in Progress in Photosynthesis Research (Metzner, H., ed.) Vol. II, p. 883–898, H. Laupp, Tubingen
16 Bennoun, P. (1970) Biochim. Biophys. Acta 216, 357–363
17 Lavorel, J. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.) pp. 223–317, Academic Press, New York
18 Joliot, P., Joliot, A., Bouges, B. and Barbieri, G. (1971) Photochem. Photobiol. 14, 287–305
19 Avron, M. (1960) Biochim. Biophys. Acta 40, 257–272
20 Bennoun, P. (1971) Thesis, Faculté des Sciences, Paris
21 Forbush, B., Kok, B. and McGloin, M. P. (1971) Photochem. Photobiol. 14, 307–321
22 Thorne, S. W. and Boardman, N. K. (1971) Biochim. Biophys. Acta 234, 113–125
23 Joliot, P. (1965) Biochim. Biophys. Acta 102, 116–134
24 Mehler, A. (1951) Arch. Biochem. Biophys. 34, 339–351
25 Stiehl, H. H. and Witt, H. T. (1969) Z. Naturforsch. 24b, 1588–1598
26 Bouges-Bocquet, B. (1975) in Proceedings of the IIIrd International Congress on Photosynthesis (Avron, M., ed.) pp. 579–588, Elsevier, Amsterdam
27 Schreiber, U. and Vidaver, W. (1975) Biochim. Biophys. Acta 387, 37–51
28 Lemasson, C. and Barbieri, G. (1971) Biochim. Biophys. Acta 245, 386–397
29 Velthuys, B. R. (1976) Thesis, Rijksuniversiteit, Leiden
30 Baxendale, J. H. and Hardy, H. R. (1953) Trans. Faraday Soc. 49, 1433–1437
31 Burstein, E. and Davidson, A. W. (1941) Trans. Electrochem. Soc. 80, 175
32 Michaelis, L., Schubert, M. P., Reber, R. K., Kuck, J. A. and Granick, S. (1938) J. Am. Chem. Soc. 60, 1678–1683
33 Bensasson, R. and Land, E. J. (1973) Biochim. Biophys. Acta 325, 175–181
34 Stiehl, H. H. and Witt, H. T. (1968) Z. Naturforsch. 23b, 220–224
35 Van Gorkom, H. J. (1976) Thesis, Rijksuniversiteit, Leiden
36 Fowler, C. F. and Kok, B. (1974) Biochim. Biophys. Acta 357, 299–307
37 Bridge, N. K. and Porter, G. (1958) Proc. Roy. Soc. Ser. A 244, pp. 259, 276
38 Land, E. J. and Swallow, A. J. (1970) J. Biol. Chem. 245, 1890–1894
39 Baxendale, J. D. and Hardy, H. R. (1953) Trans. Faraday Soc. 49, 1140–1144
40 Ausländer, W. and Junge, W. (1974) Biochim. Biophys. Acta 357, 285–298
41 Fowler, C. F. and Kok, B. (1976) Biochim. Biophys. Acta 423, 510–523

tion reaction.

Bensasson and Land [33] have demonstrated a general similarity between the plastosemiquinone anion - plastoquinone difference spectrum and that observed by Stiehl and Witt [34] for Q^-. Van Gorkom [35] has further shown, through spectral evidence, in deoxychoyate particles, that Q^- does not become protonated even down to pH 4. In agreement with these results, Fowler and Kok [36] observed no light induced proton uptake upon excitation of Photosystem II in the presence of DCMU. Furthermore, Pulles et al. [10] proposed that in chloroplasts at pH 7.8, B^- also remains unprotonated. The pK_a of durosemiquinone in ethanol-water mixtures is 5.9 [37] while that of ubisemiquinone in methanol is 6.45 [38]. These would tend to be more acid still in a more polar medium. Consequently, a simple way to explain equilibrium K_2 in favor of an electron transfer from Q to B is that B lies in a more polar environment (possibly close to the outer face of the membrane) than Q at a pH exceeding its pK_a.

Once Q becomes reduced a second time, the dismutation is favored (high K_3) as the two pK_a for plastohydroquinone (durohydroquinone, $pK_a = 11.35$ (18.8 °C) and $pK_{a2} = 12.88$ (22.5 °C) [39]) exceed the physiological pH. This model, as do the data of Pulles et al. [10], would predict proton uptake by Photosystem II on alternate flashes for previously dark adapted chloroplasts. Fowler and Kok [36] however, do not see an oscillation of period 2 for proton uptake by Photosystem II under these conditions. Thus there remains some uncertainty in the mechanism of proton uptake on the acceptor side of Photosystem II.

The relatively high equilibrium constants K_2 and K_3, while coherent with the elevated Q/A equilibrium observed in the dark, do not explain the fact that a low equilibrium constant is observed in the light. Ausländer and Junge [40] and Fowler and Kok [41] have shown that proton uptake from the outside of the thylakoid membrane is limited by a diffusion barrier ($t_{\frac{1}{2}} \cong 60$ ms, total relaxation $\cong 400$ ms) [40]. One might imagine that upon rapid (with respect to proton movement through the diffusion barrier) turnover of Photosystem II, and a consequent light induced alkalinization of the intramembrane space, protected by the diffusion barrier, protonation of B^{2-} might become kinetically and thermodynamically limited. Consequently, equilibrium $Q^-B^- \rightleftharpoons QB^{2-}$ would be shifted to the left. If such were the case, then the addition of uncouplers or illumination at low light intensity should maintain a high value for K_3 in the light.

Further simultaneous characterization of electron flow and proton uptake on the system II acceptor side might clarify the equilibrium problem and that of the actual site of protonation (whether B or A).

ACKNOWLEDGEMENTS

The author gratefully acknowledges the support of the Helen Hay Whitney Foundation and of the Commissariat à l'Energie Atomique (Bourse Joliot-Curie).

REFERENCES

1 Joliot, A. (1968) Physiol. Vég. 6, 235–254
2 Forbush, B. and Kok, B. (1968) Biochim. Biophys. Acta 162, 243–253
3 Malkin, S. (1971) Biochim. Biophys. Acta 234, 415–427

4 Radmer, R. and Kok, B. (1973) Biochim. Biophys. Acta 314, 28–41
5 Velthuys, B. R. and Amesz, J. (1973) Biochim. Biophys. Acta 325, 126–137
6 Van Gorkom, H. J. (1974) Biochim. Biophys. Acta 347, 439–442
7 Joliot, P. (1965) Biochim. Biophys. Acta 102, 116–134
8 Bouges-Bocquet, B. (1973) Biochim. Biophys. Acta 314, 250–256
9 Velthuys, B. R. and Amesz, J. (1974) Biochim. Biophys. Acta 333, 85–94
10 Pulles, M. P. J., Van Gorkom, H. J. and Willemsen, J. G. (1976) Biochim. Biophys. Acta, 449, 536–540
11 Diner, B. (1975) in Proceedings of the IIIrd International Congress on Photosynthesis (Avron, M., ed.), pp. 589–601, Elsevier, Amsterdam
12 Diner, B. and Mauzerall, D. (1973) Biochim. Biophys. Acta 305, 329–352
13 Kok, B., Forbush, B. and McGloin, M. P. (1970) Photochem. Photobiol. 11, 457–475
14 Van Best, J. A. and Duysens, L. N. M. (1975) Biochim. Biophys. Acta 408, 154–163
15 Lavorel, J. (1969) in Progress in Photosynthesis Research (Metzner, H., ed.) Vol. II, p. 883–898, H. Laupp, Tubingen
16 Bennoun, P. (1970) Biochim. Biophys. Acta 216, 357–363
17 Lavorel, J. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.) pp. 223–317, Academic Press, New York
18 Joliot, P., Joliot, A., Bouges, B. and Barbieri, G. (1971) Photochem. Photobiol. 14, 287–305
19 Avron, M. (1960) Biochim. Biophys. Acta 40, 257–272
20 Bennoun, P. (1971) Thesis, Faculté des Sciences, Paris
21 Forbush, B., Kok, B. and McGloin, M. P. (1971) Photochem. Photobiol. 14, 307–321
22 Thorne, S. W. and Boardman, N. K. (1971) Biochim. Biophys. Acta 234, 113–125
23 Joliot, P. (1965) Biochim. Biophys. Acta 102, 116–134
24 Mehler, A. (1951) Arch. Biochem. Biophys. 34, 339–351
25 Stiehl, H. H. and Witt, H. T. (1969) Z. Naturforsch. 24b, 1588–1598
26 Bouges-Bocquet, B. (1975) in Proceedings of the IIIrd International Congress on Photosynthesis (Avron, M., ed.) pp. 579–588, Elsevier, Amsterdam
27 Schreiber, U. and Vidaver, W. (1975) Biochim. Biophys. Acta 387, 37–51
28 Lemasson, C. and Barbieri, G. (1971) Biochim. Biophys. Acta 245, 386–397
29 Velthuys, B. R. (1976) Thesis, Rijksuniversiteit, Leiden
30 Baxendale, J. H. and Hardy, H. R. (1953) Trans. Faraday Soc. 49, 1433–1437
31 Burstein, E. and Davidson, A. W. (1941) Trans. Electrochem. Soc. 80, 175
32 Michaelis, L., Schubert, M. P., Reber, R. K., Kuck, J. A. and Granick, S. (1938) J. Am. Chem. Soc. 60, 1678–1683
33 Bensasson, R. and Land, E. J. (1973) Biochim. Biophys. Acta 325, 175–181
34 Stiehl, H. H. and Witt, H. T. (1968) Z. Naturforsch. 23b, 220–224
35 Van Gorkom, H. J. (1976) Thesis, Rijksuniversiteit, Leiden
36 Fowler, C. F. and Kok, B. (1974) Biochim. Biophys. Acta 357, 299–307
37 Bridge, N. K. and Porter, G. (1958) Proc. Roy. Soc. Ser. A 244, pp. 259, 276
38 Land, E. J. and Swallow, A. J. (1970) J. Biol. Chem. 245, 1890–1894
39 Baxendale, J. D. and Hardy, H. R. (1953) Trans. Faraday Soc. 49, 1140–1144
40 Ausländer, W. and Junge, W. (1974) Biochim. Biophys. Acta 357, 285–298
41 Fowler, C. F. and Kok, B. (1976) Biochim. Biophys. Acta 423, 510–523