Effect of preillumination on the P-680$^+$ reduction kinetics in chloride-free photosystem II membranes

Taka-aki Ono*, Helene Conjeaud, Hermann Gleiter*, Yorinao Inoue* and Paul Mathis

Service de Biophysique, Département de Biologie, Centre d'Études Nucléaires de Saclay, 91191 Gif-sur-Yvette, France and **Solar Energy Research Group, The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-01, Japan*

Received 30 May 1986

The re-reduction course of P-680$^+$, the photooxidized PS II primary donor, was measured as a function of excitation number in Cl^--depleted PS II membranes. After the 1st and 2nd excitations the signal amplitude of P-680$^+$ is small, indicating a submicrosecond reduction of P-680$^+$ by Z, the secondary donor of PS II. After the 3rd excitation, however, a larger P-680$^+$ signal with a 40–50 μs half-life is observed. The slow decay of this signal is attributed to a back-reaction with a reduced acceptor in the presence of the Z^+S_2 state on the donor side. The state Z^+S_2 has a lifetime longer than 300 ms and its formation was found to depend on the presence of the abnormal S_2 state created by the 1st excitation. The P-680 data and thermoluminescence measurements show that the S-state advancement beyond S_2 is blocked in the absence of Cl^- and that the Cl^--free abnormal S_2 state has a lifetime about 10-times longer than the normal S_2 state.

Oxygen evolution *S-state transition* *Cl^-* *P-680* *Thermoluminescence* *Photosystem II membrane*

1. INTRODUCTION

Cl^- is an indispensable cofactor for photosynthetic O_2 evolution [1]. A study by Izawa et al. [2] was the first to provide an indication that the functional site of Cl^- is in the water-oxidation enzyme. Recent developments in protein chemistry of the water oxidase system have confirmed this view. In particular it was proposed that the role of the two extrinsic proteins (24 and 16 kDa) was to afford high-affinity binding sites for Cl^- in the vicinity of the water-oxidation center [3,4]. Since the finding of Muallem et al. [5] that the Cl^--depleted water oxidase is able to accumulate some oxidized equivalents, various approaches have been employed to investigate the S-state turnover in Cl^--depleted PS II, e.g. by means of fluorescence quenching by P-680$^+$ [6,7], flash O_2 yield measurements [8] or thermoluminescence oscillation [9,10]. The results are largely consistent with respect to the fact that one or two oxidized equivalents can be accumulated on the donor side of the reaction center, but there remain some ambiguities as to the precise site of inhibition in the S-state turnover.

Here, we report a direct spectroscopic measurement of P-680$^+$ re-reduction kinetics in Cl^--depleted PS II membranes after the 1st, 2nd and 3rd flash excitation. The results show that two positive equivalents are stored, presumably in the state S_2Z^+, and then permit the observation of long-lived P-680$^+$ after the 3rd flash. Measurements of P-680$^+$ and thermoluminescence show that the state induced by the 1st flash (presumably a modified S_2) lasts much longer than the normal S_2 state.

Abbreviations: PS, photosystem; Mes, 4-morpholineethanesulfonic acid; Chl, chlorophyll; Q_A, primary quinone acceptor of PS II; Q_B, secondary quinone acceptor of PS II

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

2. MATERIALS AND METHODS

Spinach PS II membranes capable of O_2 evolution [11] were prepared as in [12] and stored in liquid N_2. After thawing, the membranes were suspended in 0.4 M sucrose/20 mM NaCl/40 mM Mes-NaOH (pH 6.5) and centrifuged (35000 × *g*, 10 min). The pelleted membranes were resuspended in a low Cl^- medium, 0.4 M sucrose/2 mM NaCl/4 mM Mes-NaOH (pH 6.5), after two washes with the same low Cl^- medium. After a 2 h dark relaxation at 0°C, the following treatments were carried out under dim green safe light: Cl^- depletion was accomplished by SO_4^{2-} replacement [10] by diluting the membranes (18 μl, 3 mg Chl/ml) with 2 ml of 0.4 M sucrose/50 mM Na_2SO_4/40 mM Hepes-NaOH (pH 7.5), and the samples were subjected to measurement after 2 min incubation in the dark at room temperature. Cl^- repletion was done by readdition of NaCl to the depleted membranes at a final concentration of 50 mM. Cl^--sufficient control particles were obtained by diluting the low Cl^- particle suspension with a high Cl^- medium, 0.4 M sucrose/50 mM NaCl/40 mM Hepes-NaOH (pH 7.5).

The re-reduction kinetics of $P\text{-}680^+$ were measured spectroscopically at room temperature as in [13]. The membranes (30 μg Chl/ml) were excited with an actinic laser flash at 595 nm from a dye laser pumped by a frequency-doubled Nd/YAG laser (Quantel, duration 15 ns) and the absorption change at 820 nm was recorded. The actinic laser flash was eventually preceded, as described in section 3, by a xenon flash (Stroboslave, white, 5 μs) and/or a variable number of preilluminating flashes from the same laser. All flashes were saturating for PS II reactions. For the measurement of ΔA, we used the same instrument as described in [14]. The optical path of the cuvette was 10 mm, and 0.2 mM 2,5-dimethylquinone was added as an electron acceptor.

Thermoluminescence glow curves were measured as described [12,15]. The membranes (0.2 mg Chl/ml) were excited with a saturating xenon flash (Sugawara, MS-230, white, 5 μs) at room temperature, cooled quickly or after various periods of dark incubation at room temperature, and then the light emission during warming (1°C/s) was recorded against the sample temperature.

3. RESULTS AND DISCUSSION

The effect of Cl^- depletion on the reduction kinetics of $P\text{-}680^+$ was measured by the absorption change at 820 nm. In a first series of experiments, PS II membranes were excited by a laser flash which was preceded by 0–3 laser flashes with a time interval of 10 s. In both Cl^--sufficient and Cl^--deficient membranes, only a small signal was observed decaying in the microsecond time range (5–20 μs), accompanied by a minor component with a much longer lifetime as in fig.1, lanes a,b, first flash. The signal amplitude showed no dependence on the excitation number (not shown). The amplitude of the ΔA corresponds to about 15% of the total photooxidizable P-680, which was measured after Tris treatment of the membranes: $P\text{-}680^+$ then decays with a $t_{1/2}$ of 8 μs at pH 6.0 (in agreement with [16]) and corresponds to one $P\text{-}680^+$ per 240 chlorophyll molecules, assuming an extinction coefficient of 7000 $M^{-1} \cdot cm^{-1}$ at 820 nm. These results are in agreement with the reports that about 80% of $P\text{-}680^+$ is reduced within the submicrosecond time range by the elec-

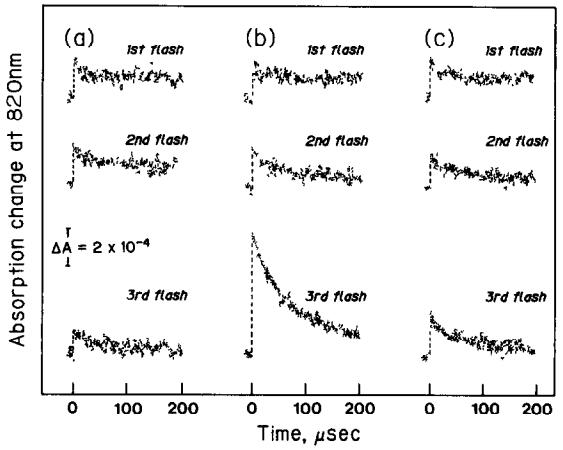

Fig.1. Effect of Cl^- depletion and repletion on the 820 nm absorption change induced in PS II membranes on excitation with a laser flash. Excitation regimes: 1st flash, single laser flash; 2nd flash, a 5 μs xenon flash – 50 ms – single laser flash; 3rd flash, a laser flash – 10 s – a 5 μs xenon flash – 50 ms – single laser flash. The absorption change was recorded for the final laser flash. Each trace was recorded using a fresh sample (30 μg Chl/ml). (a) Control particles, (b) Cl^--depleted particles, (c) Cl^--replete particles.

tron donation from the water-oxidizing system, while the remaining 20% is slowly reduced within the microsecond time range [17,18]. The microsecond phase of P-680$^+$ reduction is thus little influenced by the S states. The data also show that Cl^-depletion does not impair the coupling of Z with P-680, as already proposed on the basis of fluorescence measurements [6].

Assuming that the donor side of PS II is essentially constituted of a short linear sequence comprising the S state complex, Z and P-680, we rationalized that counting the number of stored oxidizing equivalents would require an additional flash fired before the last laser flash, in order to keep Z oxidized. The result of such an experiment is shown in fig.1, 2nd and 3rd flash. In Cl^--depleted PS II (lane b) a large signal is observed when the last laser flash is preceded by both one xenon flash, given 50 ms before, and one laser flash, given 10 s before. This result is interpreted as follows: the first flash induces the S_1 to S_2 transition and the 2nd flash oxidizes Z which is not yet re-reduced when the last flash is fired, permitting observation of a slow decay of P-680$^+$ ($t_{1/2}$ = 40–50 μs). This slow decay takes place in 60–70% of the PS II centres. It is implied that, in Cl^--depleted PS II, Z^+ cannot oxidize S_2, perhaps because Cl^- is required for the S_2 to S_3 transition. This is in agreement with the fluorescence data of Itoh et al. [6] and Theg et al. [7] and with recent EPR measurements [19]. The large P-680$^+$ signal is observed when the xenon flash is preceded by 1–3 laser flashes, with a time interval of 10 s, but not when there is no laser flash before the xenon flash. This indicates that the oxidation of S_1 by Z^+ is faster than 50 ms. The depletion of Cl^- is responsible for the change of the P-680$^+$ reduction kinetics, since the large 40–50 μs signal was absent in the Cl^--sufficient control (fig.1a) and since the repletion of Cl^- could nearly restore the behaviour of the control (fig.1c).

As shown in fig.2, the 40–50 μs phase of the P-680$^+$ reduction kinetics, in Cl^--depleted PS II, is still markedly induced (50% of the maximum) when the time interval between the 2nd and 3rd flashes is 300 ms instead of 50 ms. A preliminary set of experiments with a variable interval between the 2nd and 3rd excitations indicated that Z^+ was reduced in a biphasic manner, about half in the hundreds of milliseconds domain and about half in the seconds domain, in reasonable agreement with the results of Itoh et al. [6]. From this, it may be deduced that the electron donation from S_2 to Z^+ is shut off or greatly slowed down in the absence of Cl^-.

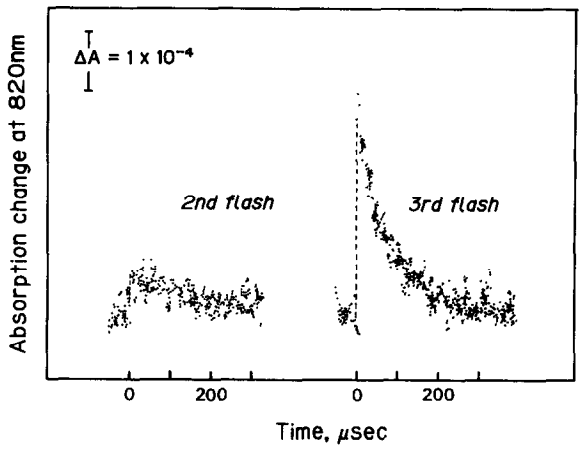

Fig.2. Effect of an increased interval between the 2nd and 3rd excitations on the 820 nm absorption change in Cl^--depleted PS II membranes. For the 2nd excitation, two laser flashes were fired 300 ms apart. For the 3rd excitation, three laser flashes were fired 1 s and 300 ms apart between the 1st and 2nd and between the 2nd and 3rd flashes, respectively. Each trace was recorded using a fresh sample (30 μg Chl/ml).

On the basis of thermoluminescence and EPR measurements, it has been shown that, in Cl^--depleted PS II, the S_2 state has unusual properties: great stability (as shown by its long lifetime and the appearance of the thermoluminescence band at 42°C instead of 30°C) and abnormal EPR properties [9,19]. Since the large and slow P-680$^+$ signal after the 3rd excitation of Cl^--depleted membranes observed in the present study results from an excitation in the presence of the Z^+S_2 state on the donor side, we may expect that the amplitude of the P-680$^+$ signal after the 3rd excitation will depend on the number of the centers having the abnormal S_2 state after the 1st excitation. To check this hypothesis we changed the time interval between the 1st and 2nd flashes and measured the amplitude of the P-680$^+$ signal induced by the 3rd flash in Cl^--depleted PS II (fig.3, □). We found that the state induced by the 1st flash is long-lived ($t_{1/2}$ ~5 min) and decays like the thermoluminescence amplitude which probes the

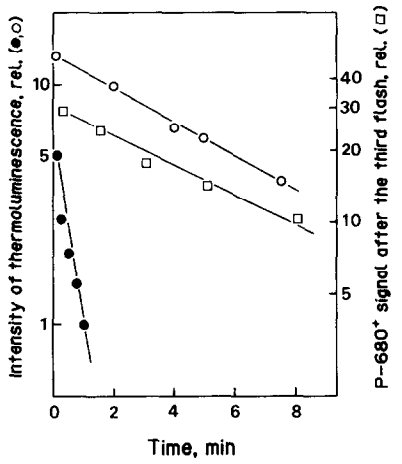

Fig.3. Dependence of the 3rd flash excited P-680$^+$ signal on the interval between the 1st and 2nd excitations, as compared with the deactivation course of the S_2 state. P-680$^+$ was measured after the 3rd excitation as in fig.1 by changing the interval between the 1st and 2nd flashes (□). Each point was measured with a fresh sample of Cl^--depleted PS II membranes (30 μg Chl/ml). Deactivation of S_2 state was measured from the height of thermoluminescence band which arises from the charge pair ($S_2Q_B^-$) remaining after various dark incubation times (room temperature) between the flash excitation and the subsequent cooling to 77 K; PS II membranes were Cl^--depleted (○) or Cl^--sufficient (●).

$S_2Q_B^-$ state (fig.3, ○). The state $S_2Q_B^-$ decays much faster in a Cl^--sufficient control (fig.3, ●).

In conclusion, this study has demonstrated that only one turnover from S_1 to S_2 is allowed in the Cl^--depleted O_2-evolving center and the resulting Cl^--free S_2 state is abnormally stable in comparison with the Cl^--sufficient S_2 state. This indicates that Cl^- works to maintain the redox property of S_2 to guarantee the normal S-state transitions. We have not precisely discussed the reason why P-680$^+$ decays with a $t_{1/2}$ of 40–50 μs when Z is oxidized. It has been previously shown in Tris-treated chloroplasts that, when Z is oxidized, P-680$^+$ decays via a back-reaction with Q_A^-, with $t_{1/2}$ of 130 μs [18,20]. It is quite possible that the 40–50 μs decay observed here is also due to a back-reaction with Q_A^-; the difference in kinetics may originate in the Tris treatment which, presumably, also weakens the coupling between Z and P-680. Although a decisive conclusion on this point awaits simultaneous measurements of P-680$^+$ reduction and Q_A^- oxidation in Cl^--depleted samples, the idea appears probable in view of the close structural relationships between the photochemical center of PS II and the water-oxidation center [21]. Alternatively, the 40–50 μs phase could be identical to the 35 μs phase reported by Renger and co-workers and attributed to a back-reaction between P-680 and an electron acceptor different from Q_A [22,23]. In our hands the Cl^- depletion, as studied here, provided the first example of a slow reduction of P-680$^+$ under conditions where a good coupling is maintained between the donor Z and P-680.

ACKNOWLEDGEMENTS

This study was supported at CEN Saclay by the CEA (France) and by a grant for Solar Energy Conversion by Means of Photosynthesis at the Institute of Physical and Chemical Research (RIKEN) given by Science and Technology Agency (Japan). T.O. was supported at CEN Saclay by a grant based on the Versailles Summit Cooperation Program on Photosynthesis and Photoconversion. H.G. of the University of Tübingen (FRG) was a guest at RIKEN supported by a scholarship from the Studienstiftung des Deutschen Volkes. Helpful discussions with Dr A.W. Rutherford are also acknowledged.

REFERENCES

[1] Critchley, C. (1985) Biochim. Biophys. Acta 811, 33–46.
[2] Izawa, S., Heath, R.L. and Hind, G. (1969) Biochim. Biophys. Acta 180, 388–389.
[3] Andersson, B., Critchley, C., Ryrie, I.J., Jansson, C., Larsson, C. and Anderson, J.M. (1984) FEBS Lett. 168, 113–117.
[4] Akabori, K., Imaoka, A. and Toyoshima, Y. (1984) FEBS Lett. 173, 36–40.
[5] Muallem, A., Farineau, J., Laine-Böszormenyi, M. and Izawa, S. (1981) in: Photosynthesis (Akoyunoglou, G. ed.) vol.II, pp.435–443, Balaban, Philadelphia.
[6] Itoh, S., Yerkes, C.T., Koike, H., Robinson, H.H. and Crofts, A.R. (1984) Biochim. Biophys. Acta 766, 612–622.
[7] Theg, S.M., Jursinic, P. and Homann, P.H. (1984) Biochim. Biophys. Acta 766, 636–646.
[8] Sinclair, J. (1984) Biochim. Biophys. Acta 764, 247–252.

[9] Homann, P.H. and Inoue, Y. (1986) in: Ion Interactions in Energy Transfer Biomembranes (Papageorgiou, G.C. et al. eds) pp.279–290, Plenum, London.
[10] Homann, P.H., Gleiter, H., Ono, T. and Inoue, Y. (1986) Biochim. Biophys. Acta, in press.
[11] Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234.
[12] Ono, T. and Inoue, Y. (1985) Biochim. Biophys. Acta 806, 331–340.
[13] Kramer, H. and Mathis, P. (1980) Biochim. Biophys. Acta 593, 319–329.
[14] Mathis, P., Butler, W.L. and Satoh, K. (1979) Photochem. Photobiol. 30, 603–614.
[15] Rutherford, A.W., Crofts, A.R. and Inoue, Y. (1982) Biochim. Biophys. Acta 682, 457–465.
[16] Reinman, S., Mathis, P., Conjeaud, H. and Stewart, A. (1981) Biochim. Biophys. Acta 635, 429–433.
[17] Van Best, J.A. and Mathis, P. (1978) Biochim. Biophys. Acta 503, 178–188.
[18] Brettel, K. and Witt, H.T. (1984) Photobiochem. Photobiophys. 6, 253–260.
[19] Ono, T., Zimmermann, J.L., Inoue, Y. and Rutherford, A.W. (1986) Biochim. Biophys. Acta, in press.
[20] Conjeaud, H., Mathis, P. and Paillotin, G. (1979) Biochim. Biophys. Acta 546, 280–291.
[21] Ikeuchi, M., Yuasa, M. and Inoue, Y. (1985) FEBS Lett. 185, 316–322.
[22] Gläser, M., Wolff, C. and Renger, G. (1976) Z. Naturforsch. 31c, 712–721.
[23] Eckert, H.J. and Renger, G. (1980) Photochem. Photobiol. 31, 501–511.