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A binding site for chloride in photosystem II which specifically influences acceptor-side electron transfer and plastoquinone binding

L-E Andréasson[1], K Olesen[1], Z Deák[2] , K Lindberg[1], I Vass[2]

[1]*Department of Chemistry, Division of Biochemistry and Biophysics, Göteborg University, P.O. Box 462, SE-405 30 Göteborg, Sweden and* [2]*Institute of Plant Biology, Biological Research Center of the Hungarian Academy of Sciences, P.O. Box 521, H-6701 Szeged, Hungary*

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Introduction

A considerable amount of spectroscopic and kinetic evidence accumulated over the years places the site of action of Cl^- on the PS II donor side. Comparably little is known about the influence of Cl^- on acceptor-side function. Measurements of thermoluminescence and luminescence, imply that properties of the charge separated state and the rate of charge recombination may affected by Cl^- depletion (Vass et al. 1987) although this seems to be dependent of factors including the properties of the medium (Krieger et al. 1998, Homann 1999). These effects have, however, generally been interpreted as donor-side effects, e.g. a stabilization of the S_2 state due to a decrease in its midpoint potential rather than a corresponding shift in the potential of the Q_A/Q_A^- pair in the opposite direction, although the later possibility cannot be excluded. The present study is an attempt to clarify the role of Cl^- in acceptor-side function using chlorophyll fluorescence to monitor electron transfer events on the acceptor side of PS II. Our studies show that Cl^- may have a role in regulating the electron flow between the primary and secondary plastoquinones.

Materials and methods

Cl^--depletion of PS II membranes and preparation of PS II membranes with 1 Cl^-/PS II was carried out as described earlier (Lindberg et al. 1996). Flash-induced induction and decay of chlorophyll fluorescence was measured at room temperature and a chlorophyll concentration of 15 µg/ml with a PAM fluorimeter (Walz, Effeltrich) (Vermaas et al.1994) as an average of 10 individual traces obtained with a flash spacing of 20 s. Alternatively, fluorescence decay kinetics after giving a single excitation flash to dark-adapted PS II samples were measured with a double-modulation fluorimeter (PSI Instruments, Brno) (Vass et al. 1999). The first measurement of the flash-induced fluorescence was taken 150 µs after the flash. The decay kinetics of the fluorescence-yield was analyzed by least-square curve fitting using two exponential and one hyperbolic component (Vass et al. 1999) with free-floating parameters:

$$F(t) - F_o = A_1 \exp(-t/T_1) + A_2 \exp(-t/T_2) + A_3/(1+t/T_3)$$

where $F(t)$ is the variable fluorescence yield, F_o is the basic fluorescence before the flash, $A_{(1-3)}$ are the amplitudes of the fluorescence decay components and $T_{(1-3)}$ are their respective time constants. The Joliot model (Joliot & Joliot 1964) was used to correct for the non-linear correlation between fluorescence yield and the redox state of Q_A, using with a value of 0.5 for the energy-transfer parameter between PS II units.

All experiments were carried out in 20 mM Mes-NaOH, pH 6.3, with 0.4 M sucrose or 1.4 M glycerol as cryoprotectant.

Results and discussion

An actinic microsecond flash, given to PS II membranes in a Cl^--sufficient medium containing sucrose, resulted in a virtually instantaneous rise in chlorophyll fluorescence due to the closing of the traps after Q_A reduction, followed by a multiphasic decay of the fluorescence as Q_A^- is re-oxidized (Fig 1). The analysis of the decay of induced fluorescence is based on the widely used model of the two-electron gate (Renger et al. 1995, Crofts & Wraight 1997). According to the model, the fast decay phase (about 1 ms, relative amplitude 32 %) originates from electron transfer to Q_B, a 15 ms phase (amplitude 30 %) most likely results from electron transfer to the secondary quinone in centers where the Q_B site is empty at the time of the actinic flash. The slow phase in the time range of seconds arises from recombination of the electron with the donor side. With the Q_B inhibitor DCMU in the Q_B site only a monophasic fluorescence decay with a halftime of about 1 s was observed (Fig. 1), indicating that Q_A^- oxidation occurs only by slow charge recombination with the S_2 state of the donor side.

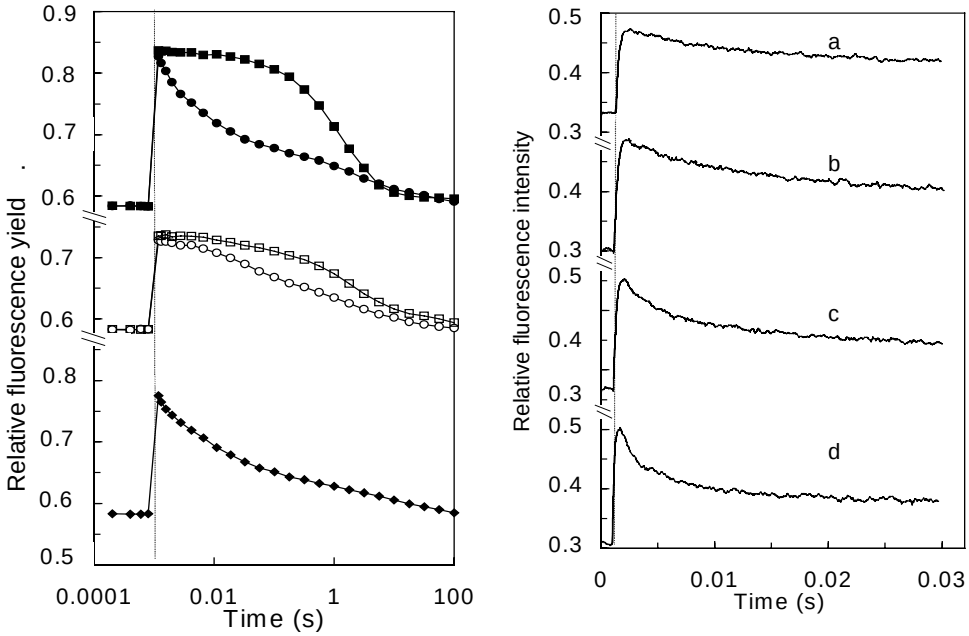

Fig. 1. The effect of Cl^- depletion/re-addition on the decay of flash-induced chlorophyll fluorescence PS II membranes. Measurements performed with the double-modulation fluorimeter. (•) Control (25 mM Cl^-); (■) control + 10 µM DCMU; (○) Cl^--depleted; (□) Cl^- -depleted + 10 µM DCMU; (◆) Cl^--depleted reconstituted with 25 mM NaCl. All treatments prior to the measurements were done in darkness. The actinic flash was given at the position indicated by the vertical line.

Fig. 2. The effect of Cl^- binding on the decay of flash-induced chlorophyll fluorescence in PS II membranes. The traces represent: a, membranes equilibrated and measured in 10 µM Cl^-; b, PS II membranes with 1 Cl^- bound/PS II (se text) in Cl^--free buffer; c, PS II membranes with 1 Cl^- bound/PS II in 25 mM Cl^-; d, control membranes in 25 mM Cl^-. The buffer used in the experiment contained 0.4 M sucrose. All treatments of the membranes prior to the measurements were done in darkness. The traces have been displaced vertically for clarity.

In PS II membranes, where bound Cl^- had been completely removed by dialysis the 1 ms and 15 ms phases were replaced by two new phases with halftimes ≈13 and 86 ms, respectively, and relative contributions to the total amplitude of 20 and 14 %, respectively (Fig. 1). The halftime of the slow charge recombination phase did not

change but its relative amplitude increased (66 %). These results indicate that forward transfer of the electron from Q_A^- is still possible after Cl^- depletion although at a reduced rate. Secondly, the amplitude of the fluorescence rise after the flash was significantly lower (about 30%) than in control membranes or DCMU-treated membranes in the presence of chloride. The lower amplitude indicates that in Cl^--depleted PS II membranes the recombination of the electron on Q_A^- (or pheophytin) with the oxidized primary donor $P680^+$ successfully competes with electron transfer to $P680^+$ from the PS II donor side. This effect is expected if the electron transfer from the PS II donors takes place at lower rates in the absence of Cl^- and is fully in accordance with our observation that PS II membranes, in which Cl^- has been removed by dialysis, show partially inhibited steady-state oxygen evolution (Lindberg et al. 1996) and slower rise of the induced fluorescence (not shown). Both effects of Cl^- depletion, i.e. the disappearance of the 1 ms fluorescence decay and the lower amplitude of the fluorescence rise, are reversible and normal behavior was largely restored when Cl^- was added back to the depleted PS II membranes (Fig 1).

The slower halftimes of the fast and middle decay phases of the fluorescence yield after Cl^- depletion compared to those observed with Cl^- present suggest that both the rate of Q_A^- to Q_B electron transfer and the binding of plastoquinone to the empty Q_B site become slower in the absence of Cl^-. In addition, the dissociation constant for plastoquinone appears to increase. This is indicated by the decreased relative amplitude of the new fast phase compared to that of the original (20 and 32 %, respectively) which suggests that the amount of plastoquinone initially bound is lower than with Cl^- present. Moreover, the smaller amplitude of the new middle phase implies that the extent of binding of plastoquinone to the empty site is also decreased.

In 1 Cl^-/PS II membranes (Lindberg et al. 1996), the rapid, millisecond decay phases involving electron transfer to Q_B and to the plastoquinone pool, were replaced by significantly slower kinetics. However, the amplitude of the initial fluorescence rise was the same as in membranes in the presence of high concentrations of chloride (cf. Fig. 2 b, d). When the kinetics of the induced fluorescence after a flash in the 1 Cl^-/PS II samples were measured in the presence of 1 mM or higher concentrations of Cl^-, the rapid kinetic phases of the fluorescence decay were largely restored (Fig. 2 c).

The observation that the amplitude of the fluorescence rise in the 1 Cl^-/PS II membranes is the same as that in Cl^--sufficient control membranes indicates that the donor-side electron transfer is not significantly inhibited which is consistent with our previous observation that the single, tightly bound chloride ion is sufficient for full oxygen-evolution activity (Lindberg et al. 1996).

Restoration of normal acceptor-side electron-transfer rates required fairly high concentrations of chloride, in the order of 1 mM, in the medium. This indicates that a site for chloride, different from that previously shown to be associated with the donor side, controls acceptor-side electron transfer in PS II. The affinity of this site for chloride was determined by analysis of the rapid fluorescence decay phases after incubation of PS II membranes for 3 h at various concentrations of chloride (Fig. 3). Long incubation times were used to ensure that the PS II binding site associated with the donor side would be in the high-affinity (K_d = 20 µM), closed state (Lindberg et al. 1996). The results of this binding experiment again emphasize the different behavior of the two binding sites. At submillimolar concentrations of Cl^- the amplitudes of the rapid decay phases of the induced fluorescence were absent but recovery occurred at concentrations around 1 mM. An estimation of the observed

dissociation constant based on the concentration dependence of the amplitude of the rapid decay phases gave a value of about 0.5 mM.

Kinetic measurements of the induced fluorescence immediately (15 s) after mixing of chloride-depleted PS II membranes with buffer containing chloride in the range 0 to 25 mM gave results similar to those obtained after long incubation with chloride (not shown). This indicates that the binding of Cl^- to the acceptor-side site is rapid. Pre-incubation for 3 h in the presence of 1 mM Cl^- followed by quick dilution in Cl^--free buffer gave the same results (measured after 15 s) as Cl^--depleted samples, demonstrating rapid dissociation of Cl^- from the acceptor side. Under such conditions the tightly bound Cl^- ion associated with the donor-side remains at its binding site due to its slow dissociation.

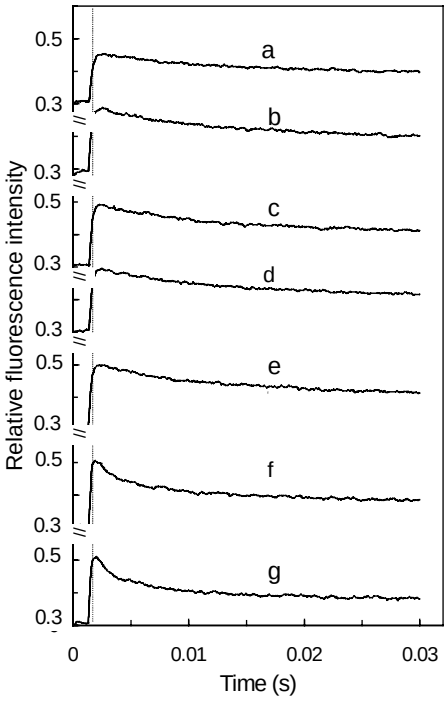

Fig. 3. Effect of Cl^- concentration on the decay of flash-induced chlorophyll fluorescence in PS II membranes. Pre-equilibration of PS II membranes for 3 h and measurements were done in the dark in 20 mM Mes-NaOH, pH 6.3, 0.4 M sucrose and the following concentrations of Cl^-: a, 10 µM; b, 25 µM; c, 50 µM; d, 100 µM; e, 250 µM; f, 1 mM; g, 25 mM. The traces have been displaced vertically for clarity.

When the cryoprotectant glycerol was exchanged for sucrose, Cl^- depletion had little effect on the decay kinetics of the induced fluorescence (not shown), in contrast to what was seen in a medium containing sucrose. Interestingly, PS II membranes depleted of Cl^- in glycerol did show slow acceptor-side electron transfer when measured in a Cl^--free sucrose buffer. Also, membranes, depleted of Cl^- in sucrose, which did not display fast acceptor-side electron transfer in a sucrose medium, did so when transferred to a Cl^--free glycerol buffer. This suggests that bound Cl^- may not be absolutely required for rapid acceptor-side electron transfer, but that compounds, such as glycerol may stabilize of the acceptor side against effects caused by Cl^- depletion.

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