Biochimica et Biophysica Acta, 325 (1973) 336–340

BBA Report

BBA 41250

Effect of oxidizing treatment on chloroplast Photosystem II reactions

RICHARD MALKIN and DAVID B. KNAFF

Department of Cell Physiology, University of California, Berkeley, Calif. 94720 (U.S.A.)

(Received September 24th, 1973)

SUMMARY

Treatment of Photosystem II chloroplast fragments with the oxidant K_2IrCl_6 causes destruction of approximately half of the chlorophyll. The treated chlorophyll-containing fragments retain Photosystem II photochemical activity and show a large light-induced free-radical signal, associated with the Photosystem II reaction-center chlorophyll, P680, but the C-550 absorbance change, associated with the Photosystem II primary electron acceptor, is absent.

Two chloroplast components related to the primary reaction of Photosystem II have recently been detected. Knaff and Arnon[1] discovered a light-induced absorbance change near 550 nm and attributed it to a chloroplast electron-transfer component which they named C-550. Evidence from several sources has related the C-550 absorbance change to the primary electron acceptor of Photosystem II (see ref. 2 for a recent review). A second light-induced change, observed by Malkin and Bearden[3], has been related to the donor side of Photosystem II. In the latter case, a light-induced electron paramagnetic resonance (EPR) free-radical signal observed at 77 °K was found to originate from the oxidized reaction-center chlorophyll ($P680^+$) of Photosystem II. The assignment of this free-radical signal to $P680^+$ was made on the basis of EPR parameters[3] and oxidation–reduction properties[4].

The question has been raised as to whether C-550 is the actual primary electron acceptor of Photosystem II or is only an indicator of the oxidation–reduction state of the true acceptor[2]. The C-550 absorbance change has been shown (1) to arise from a band shift, as opposed to a net decrease in absorbance[5], and (2) to be related to the presence of β-carotene in the chloroplast membranes[6]. These findings suggest that C-550 may not be the actual electron acceptor of Photosystem II but may reflect the oxidation–reduction

state of the primary electron acceptor. Analogous behavior for carotenoids in photosynthetic bacteria is well documented[7,8]. If C-550 acts as an indicator component, it should be possible to obtain the Photosystem II primary reaction without the presence of C-550. If, however, C-550 is obligately required for Photosystem II activity, the primary reaction of Photosystem II should not be possible without the presence of C-550.

By treatment with the strong oxidant K_2IrCl_6, Photosystem II chloroplast fragments have been prepared which have a large light-induced $P680^+$ signal and Photosystem II photochemical activity but no C-550 absorbance change. These properties are considered in terms of the primary reactants of Photosystem II.

Photosystem II chloroplast fragments (D-10), prepared by the procedure of Hauska *et al.*[9], were resuspended in 50 mM potassium phosphate buffer, pH 7.6. The reaction mixture for oxidation contained: D-10 chloroplast fragments, 0.5 mM chlorophyll; potassium phosphate buffer, pH 7.6, 50 mM; potassium ferricyanide, 25 mM; and K_2IrCl_6 (Alfa Inorganics, Beverley, Mass.), 10 mM. The K_2IrCl_6 was dissolved in 1 mM HCl and filtered just prior to use. After incubation for 5 min at room temperature, the oxidative reaction was stopped by the addition of 50 mM potassium ferrocyanide. The chloroplast fragments were reisolated by centrifugation and resuspended in 50 mM potassium phosphate buffer, pH 7.6. Chlorophyll concentrations and *a:b* ratios were determined by the method of Arnon[9].

The control D-10 Photosystem II fragments had a chlorophyll *a:b* ratio of approximately 2.0; the ratio of the oxidized fragments was approximately 1.0. Approximately half of the total chlorophyll was destroyed by K_2IrCl_6 treatment, and the decrease in the ratio of chlorophyll *a:b* appears to be related to a preferential oxidation of chlorophyll *a*.

The Photosystem II photochemical activity of the control D-10 fragments and the oxidized preparation is shown in Table I. Although the D-10 fragments can use water as

TABLE I

PHOTOSYSTEM II ACTIVITY OF CONTROL AND OXIDIZED PHOTOSYSTEM II FRAGMENTS
The reaction mixture contained: 20 mM potassium phosphate buffer, pH 7.0; 2 mM dichlorophenolindophenol (DPIP); 0.5 mM diphenylcarbazide (DPC); chloroplast fragments containing 10 μg of chlorophyll; and 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU), where present, at a concentration of $2 \cdot 10^{-5}$ M. Change in absorbance at 600 nm followed continuously with actinic illumination by 645 nm light.

Preparation	*DPC → DPIP* *μmoles/mg chlorophyll per h*
Control	168
Control + DCMU	40
Oxidized	70
Oxidized + DCMU	20

an electron donor, no water oxidation is present after oxidative treatment. Diphenylcarbazide can serve as the electron donor for dichlorophenolindophenol photoreduction in

both control and oxidized preparations. The oxidized fragments retain a substantial rate of the DPC → DPIP reaction, and this reaction is sensitive to 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (see Table I), an indication that the reaction is proceeding *via* Photosystem II.

The light-induced free-radical signal associated with the reaction-center chlorophyll of Photosystem II was present in the oxidized fragments, as shown in Fig. 1. The light-*minus*-dark EPR spectra (recorded after illumination at 40 °K in the presence of

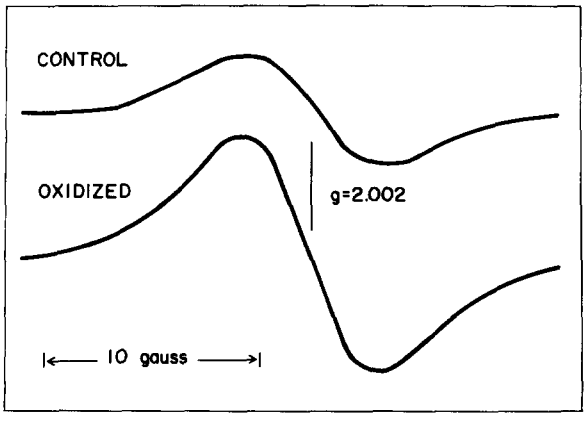

Fig.1. Light-*minus*-dark EPR spectra of free-radical signal in D-10 and oxidized D-10 fragments. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.6), 10 mM potassium ferricyanide, and D-10 or oxidized D-10 fragments (0.20 mg/ml). First-derivative EPR spectra were recorded in the dark at 40 °K prior to illumination and then after illumination for 30 s with 645-nm light. Instrument settings: frequency, 9.22 GHz; power, 0.5 mW; modulation amplitude, 2 G; scan rate, 5 G/s.

ferricyanide), indicates, on a chlorophyll basis, that the oxidized fragments are approximately 2-fold enriched in the free-radical component as compared with the control D-10 fragments.

In contrast to the free-radical component, the C-550 absorbance change (observed after illumination at 77 °K in the presence of ferricyanide) is almost completely absent in the oxidized fragments (Fig. 2). The content of C-550 (as measured by its photoreduction at 77 °K) after oxidative treatment varied from 0 to 20% of that in the untreated fragments. In these same preparations, the content of P680 (as measured by the light-induced free-radical signal) increased after oxidative treatment to values between 200 and 300% (on equal chlorophyll basis) of those found in the control samples.

The data indicate that K_2IrCl_6 preferentially oxidizes bulk chlorophyll in Photosystem II fragments and leaves the photoactive chlorophyll of the Photosystem II reaction center intact. This effect of K_2IrCl_6 on chloroplasts is similar to that previously observed with preparations of some photosynthetic bacteria[11,12].

Our findings indicate the light-induced C-550 absorbance change is considerably more sensitive to treatment with K_2IrCl_6 than is Photosystem II electron transport from

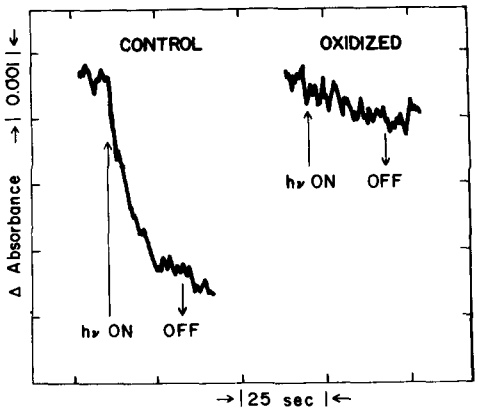

Fig.2. C-550 absorbance changes in control and oxidized D-10 fragments (548 nm *minus* 543 nm). Reaction mixture as in Fig. 1. Optical pathlength, 2 mm; temperature, 77 °K; actinic light, 660 nm. Absorbance changes measured as described in ref. 1.

diphenylcarbazide to dichlorophenolindophenol. Substantial rates of electron transport (50% of the control rates) were obtained in preparations in which no light-induced C-550 absorbance change could be detected. These results suggest that a functional C-550 is not an obligatory requirement for Photosystem II activity. Further evidence against the identity of C-550 with the primary acceptor of Photosystem II comes from the contrasting response of C-550 and P680 to K_2IrCl_6 treatment. If C-550 and P680 represent the primary reactants of Photosystem II, equivalent amounts of photoreduced C-550 and photooxidized $P680^+$ should be produced under conditions, such as those employed in this study, where no secondary reactions occur. The observation that the relative amount of P680 photooxidized increases 2- to 3-fold (on an equal chlorophyll basis) while the amount of C-550 photoreduced decreases 5- to 10-fold after treatment with K_2IrCl_6 does not appear to be compatible with C-550 being the acceptor of electrons from P680.

In summary, results reported above suggest that C-550 is not the actual primary electron acceptor of chloroplast Photosystem II and are more consistent with a role for this component as a membrane-bound indicator that responds to the oxidation–reduction state of the primary electron acceptor of Photosystem II.

We would like to thank Dr A.J. Bearden for the use of his EPR facilities during this investigation.

REFERENCES

1 Knaff, D.B. and Arnon, D.I. (1969) *Proc. Natl. Acad. Sci. U.S.* 63, 963–969
2 Butler, W.L. (1973).*Acc. Chem. Res.* 6, 177–184
3 Malkin, R. and Bearden, A.J. (1973) *Proc. Natl. Acad. Sci. U.S.* 70, 294–297.
4 Bearden, A.J. and Malkin, R. (1973) *Biochim. Biophys. Acta* in press
5 Butler, W.L. and Okayama, S. (1971) *Biochim. Biophys. Acta* 245, 237–239

6 Okayama, S. and Butler, W.L. (1972) *Plant Physiol.* 49, 769–774
7 Arnold, W. and Clayton, R.K. (1960) *Proc. Natl. Acad. Sci. U.S.* 46, 769–776
8 Dutton, P.L. (1971) *Biochim. Biophys. Acta* 226, 63–80
9 Hauska, G.A., McCarty, R.E. and Racker, E. (1970) *Biochim. Biophys. Acta* 197, 206–218
10 Arnon, D.I. (1949) *Plant Physiol.* 24, 1–15
11 Loach, P.A., Androes, G.M., Maksim, A.F. and Calvin, M. (1963) *Photochem. Photobiol.* 2, 443–454
12 Clayton, R.K. and Sistrom, W.R. (1966) *Photochem. Photobiol.* 5, 661–668