Biochimica et Biophysica Acta 933 (1988) 423–431
Elsevier

BBA 42765

Electron-transport properties of the isolated D1-D2-cytochrome *b*-559 Photosystem II reaction centre

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(Received 19 October 1987)

Key words: Photosynthesis; Photosystem II; Reaction center; Electron transport; Cytochrome *b*-559; Quinone

The electron-transport properties of the D1/D2/cytochrome *b*-559 complex, similar to that isolated by Nanba and Satoh (Proc. Natl. Acad. Sci. USA 84 (1987) 101–112), have been further characterised. Temperature-stability studies support the earlier arguments (Barber, J., et al. (1987) FEBS Lett. 220, 67–73) that silicomolybdate can be used as a convenient artificial electron acceptor for assaying the activity of this isolated Photosystem II reaction centre complex. It is also shown that although less efficient, $MnCl_2$, NH_2OH and KI can be used as electron donors in addition to diphenylcarbazide when silicomolybdate is the acceptor. When the Triton X-100 associated with the isolated complex is exchanged with beta-lauryl maltoside, a light-induced signal is observed on addition of decylplastoquinone. This signal corresponds to the photoreduction of about 30% of the cytochrome *b*-559 within the complex. A similar proportion of the cytochrome is reduced in the dark by decylplastoquinone when the reductant, sodium borohydride, is added. This result, coupled with the finding that the photoreduction of cytochrome *b*-559 is inhibited by DCMU, indicates that the added quinone is also photoreduced possibly at the Q_A and/or Q_B sites.

Introduction

The identification of the molecular sites of electron-transfer reactions in chloroplast thylakoids has depended largely on the isolation of functionally active protein complexes often with a minimum number of polypeptides. In the last few years there has been considerable success in the preparation of complexes which are essentially free of the light-harvesting chlorophyll *a/b* proteins and have full Photosystem II electron-transfer activity (for example, see Refs. 1–5). In some preparations the water-oxidation mechanism remains intact [4,5] while in others there are fewer polypeptides and the ability to evolve oxygen is lost [1–3]. Despite these advances it was not clear until recently which proteins in the PS II complex make up the reaction centre where the primary photochemical charge separation occurs. However, the recently reported isolation of a complex consisting of the D1 and D2 polypeptides (*psb*A and *psb*D gene products, respectively) and the alpha- and beta-subunits of cytochrome *b*-559 [6,7] and its characterisation as the PS II reaction centre [8–10] has fulfilled a prediction that the D1 and D2 polypeptides are structurally and functionally comparable with the L and M subunits of the reaction centre of purple bacteria [11–13]. Despite some resistance to this notion [14] there is now no

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DPC, diphenylcarbazide; Chl, chlorophyll; EDTA, ethylene diamine tetracetic acid; PS II, Photosystem II; DCIP, 2,6-dichlorophenol indophenol; Mes, 4-morpholineethanesulphonic acid; Pheo, pheophytin.

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doubt that the isolated D1/D2/cytochrome *b*-559 complex contains the chlorophylls which constitute the primary electron donor P-680 and the pheophytin molecule which acts as the primary electron acceptor. For some reason, which is not yet clear, the isolated complex appears to have no associated plastoquinone [6,15]. In the absence of a quinone acceptor, the radical pair $P\text{-}680^{+}Pheo^{-}$ produced by flash excitation of the isolated complex back-reacts with a half-time of 36 ns (Refs. 9 and 10) with a small 30 μs component due to chlorophyll triplet state formation [8,10].

Nanba and Satoh [6] were able to show that when the D1/D2/cytochrome *b*-559 complex is illuminated in the presence of excess dithionite the $P\text{-}680Pheo^{-}$ state photoaccumulated. We have confirmed this result and further shown that an optical absorption difference signal indicative of the state $P\text{-}680^{+}Pheo$ is obtained when silicomolybdate was used as an artificial electron acceptor [16]. In this paper we characterise in more detail the net electron-transport capacity of the isolated D1/D2/cytochrome *b*-559 complex with particular focus on the electron-donor side. We also report the effect of added plastoquinone on the light-induced redox properties of cytochrome *b*-559.

Materials and Methods

The D1/D2/cytochrome *b*-559 complex was isolated from the leaves of pea seedlings (*Pisum sativum* var. Feltham First) using a modified form of the method outlined previously [7,16] and which was based on the approach suggested by Nanba and Satoh [6] in which solubilisation of PS II-enriched membranes in Triton X-100 is followed by ion-exchange chromatography. Chloroplast thylakoid membranes were isolated according to a published procedure except for the use of a maceration medium of 50 mM KH_2PO_4 (pH 7.5) (NaOH), 0.35 M KCl, 0.5 mM EDTA and centrifugation at $5000 \times g$ and 4 °C for 10 min to produce a chloroplast pellet. This was resuspended in 6 mM $MgCl_2$ to rupture any intact plastids by osmotic shock and a buffer then added to give a final composition of 0.2 M sucrose, 0.1 M NaCl, 3 mM $MgCl_2$, 50 mM Tricine (pH 8.0) before further centrifugation. A PS II-enriched membrane fraction was prepared from this pellet, essentially according to Berthold et al. [17], by resuspension to a concentration of 3 mg chlorophyll per ml in a solubilisation buffer of 5 mM $MgCl_2$, 15 mM NaCl, 20 mM Mes (pH 6.3) and incubating on ice in the dark for 45 min before adding 0.5 volume 10% Triton X-100 (w/v) in solubilisation buffer. A further 30 min incubation, on ice, in the dark and centrifugation at $40000 \times g$ for 30 min gave a pellet which was resuspended to 2 mg chlorophyll per ml in a wash and storage medium of 10% (w/v) glycerol in solubilisation buffer. The membranes were pelleted once more, resuspended to 4 mg chlorophyll per ml and frozen in liquid nitrogen before storage at −80 °C.

To isolate the reaction centre complex a PS II-enriched membrane sample of 200 mg chlorophyll was washed to deplete extrinsic membrane polypeptides by dilution to 0.8 mg chlorophyll per ml in 50 mM Tris (pH 9.0), incubated on ice in the dark for 10 min and centrifuged at $40000 \times g$, 4 °C for 20 min. The pellets were resuspended in 50 mM Tris (pH 7.2) (200 ml) and 33 ml of 30% Triton X-100 added to give a final chlorophyll concentration of 0.8 $mg \cdot ml^{-1}$ and a Triton to chlorophyll ratio of 50 : 1 (w/w). A 60 min incubation with stirring, in the dark, on ice was followed by centrifugation at $100000 \times g$ for 60 min and application of the supernatant to a column (16 × 300 mm) of Fractogel TSK DEAE-650 (S) (Merck-BDH) maintained at 6 °C. Extensive washing at 0.4 $ml \cdot min^{-1}$ was carried out with 350 ml, 30 mM NaCl in a running buffer of 0.2% Triton X-100, 50 mM Tris-Cl (pH 7.2). This resulted in the return of the absorbance (at 280 nm) of the eluant to the same level as for the running buffer itself and removed more than 98% of the chlorophyll applied. The material remaining on the column was eluted by a linear concentration gradient of 2 mM NaCl per ml^{-1}. Appropriate 2 ml fractions at about 110 mM NaCl were pooled, diluted four-fold in running buffer and loaded on a smaller column (9 × 100 mm) of the same DEAE-Fractogel. After further extensive washing with 30 mM NaCl in running buffer (about 50 ml at 0.5 $ml \cdot min^{-1}$) and application of a linear NaCl gradient (5 $mM \cdot ml^{-1}$ at 0.5 $ml \cdot min^{-1}$) the complex eluted as a sharp peak (absorbance, 280 nm) at about 110 mM NaCl. For preparation of

the reaction centre in beta-lauryl maltoside the material resulting from the second column was diluted four-fold with running buffer without Triton and applied to a 9 × 30 mm DEAE-Fractogel column which had been equilibrated in a buffer containing 4 mM beta-lauryl maltoside, 50 mM Tris-Cl (pH 7.2). Extensive washing with this buffer was carried out at 0.5 $ml \cdot min^{-1}$. The complex was finally eluted with the above buffer containing 130 mM NaCl at 0.1 $ml \cdot min^{-1}$. Each preparation was checked for purity by comparison with previous determinations [16] of the optical absorbance spectrum, the cytochrome *b*-559-to-chlorophyll *a* ratio and the polypeptide composition using Coomassie Blue staining after SDS-polyacrylamide gel electrophoresis on either 12–17% gradient gels, or 10–20% gels containing 6 M urea. Chlorophyll levels were measured according to Arnon [18] and cytochrome *b*-559 estimated from reduced (dithionite) minus oxidised (ferricyanide) absorbance difference at 559 nm, taking an extinction coefficient of 15 mM^{-1} [19].

Light-induced absorbance changes were measured with a Perkin-Elmer 557 dual-beam spectrophotometer with side illumination of a 1 ml, 1 cm path length sample cuvette from a quartz-iodine source equipped with appropriate light guides and transmission filters (Calflex heat filter and 2 mm Schott RG660 cut-off filter). The intensity at the cuvette surface (300 $\mu E \cdot m^{-2} \cdot s^{-1}$) was attenuated with neutral density filters, the photomultiplier shielded by a Schott BG 38 cut-off filter and the cuvette temperature controlled to 4°C. Reduction of silicomolybdate (0.2 $mg \cdot ml^{-1}$; Pfaltz and Bauer, Inc., Stanford, CT, U.S.A.) was monitored by an increase in absorbance at 600 nm in 1 ml 50 mM Tris (pH 8.0) with a sample concentration of 2 μg chlorophyll *a* per ml^{-1}. This wavelength was chosen to monitor silicomolybdate reduction to avoid the superposition of any other light-induced signal. An appropriate extinction coefficient of 4.8 mM^{-1} was used, after determination by recording the reduced-minus-oxidised difference change at 600 nm for standards dissolved in the Tris assay medium (pH 8.0).

Results

We have previously shown that when illuminated the isolated PS II reaction-centre complex consisting of D1/D2/cytochrome *b*-559 can catalyse the net reduction of silicomolybdate with diphenylcarbazide as an electron donor [16]. We have now tested a range of compounds, previously shown to act as artificial electron donors to PS II in intact and fragmented thylakoids as possible alternatives to DPC [20–22]. In all cases the acceptor was silicomolybdate whose light-induced reduction was monitored as an increase in absorbance at 600 nm. Some of the compounds tested gave no net electron-transport activity (ascorbate and hydroquinone), while others could not be used because silicomolybdate was reduced directly without the addition of PS II reaction centre (e.g., benzidine and *p*-phenylenediamine). However, 1 mM $MnCl_2$, NH_2OH and KI all gave significant activity. A comparison of the relative rates is given in Table I and demonstrates that 1 mM DPC was the most effective donor. For DPC, rates of about 1000 micro-equivalents electrons per mg Chl per h were recorded with 2 μg Chl per ml in a cuvette illuminated at 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ and with an assay temperature of 4°C. Light-intensity curves indicated that with this low level of chlorophyll in the measuring cuvette, 300 μE·

TABLE I

LIGHT DEPENDENT REDUCTION OF SILICOMOLYBDATE BY VARIOUS DONORS

The absolute rate corresponding to 100% was estimated to be 870 ± 189 μequiv. electrons per mg per h. The values given are the average (± S.E.) of 3–5 separate preparations of the PS II reaction centre (not transferred to laurylmaltoside). Additions were 2.5 mM EDTA and 1 mM for donors.

a) Relative rates

Donor	−EDTA	+EDTA
DPC	100	101 ± 4
$MnCl_2$	48 ± 5	18 ± 5
NH_2OH	42 ± 5	20 ± 4
KI	12 ± 2	10 ± 3

b) Action of combined donors on relative rates

Donors	No. addition	+DPC	+$MnCl_2$	+NH_2OH	+KI
DPC	100	–	80 ± 19	62 ± 12	103 ± 10
$MnCl_2$	48 ± 5	80 ± 19	–	43 ± 8	40 ± 3
NH_2OH	42 ± 5	62 ± 12	43 ± 8	–	31 ± 5
KI	12 ± 2	103 ± 10	40 ± 3	31 ± 5	–

$m^{-2} \cdot s^{-1}$of light transmitted by the Schott RG660 cut-off filter was just about saturating for the net electron-transport activity. Therefore the rates are unexpectedly low and must reflect the poor ability of the artificial electron donor-acceptor systems to compete with the back-reaction between reduced pheophytin and oxidised P-680. Addition of 2.5 mM EDTA to the assay medium resulted in an inhibition of the $MnCl_2$ supported rate (see Table Ia) but there was no effect of this divalent cation chelator on the DPC and KI supported rates. It should be noted, however, that the ability of NH_2OH to donate electrons was inhibited. The reason for the latter observation is as yet unclear and could be due to a requirement of divalent cations for this reaction. The effect of adding combinations of the donors is shown in Table Ib. Despite the fact that it has been claimed that some donors (e.g., $MnCl_2$ and DPC) use different sites [20], no synergism was noted and indeed in the case of DPC and NH_2OH their joint addition caused a significant decrease when compared with the rate measured with only DPC present.

All the above measurements were made at about 4°C because exposure to higher temperatures reduced the ability of the isolated PS II reaction to catalyse the light-induced reduction of silicomolybdate. This inhibition at temperatures above 4°C was time dependent (see Fig. 1). For example, at 26°C, 50% loss of activity occurred in 4 min when the preparation was incubated in the dark before assaying its activity. Although this instability of the reaction occurred in the dark, the rate of loss of activity could be further enhanced if the sample was illuminated during the incubation period (see Fig. 1).

The sensitivity of the light-induced net reduction rate of silicomolybdate to incubation temperature above 4°C is also reflected in the inability of the PS II reaction centre to photoaccumulate reduced pheophytin in the presence of excess dithionite (data not shown). The loss of these activities seem to be due to degradation of the isolated complex, which can be monitored by a blue shift in the optical absorption and emission peaks in the red. The fact that the silicomolybdate reaction responds in this way suggests that it is a valid assay of the functional activity of the isolated D1/D2/cytochrome *b*-559 complex, a conclusion

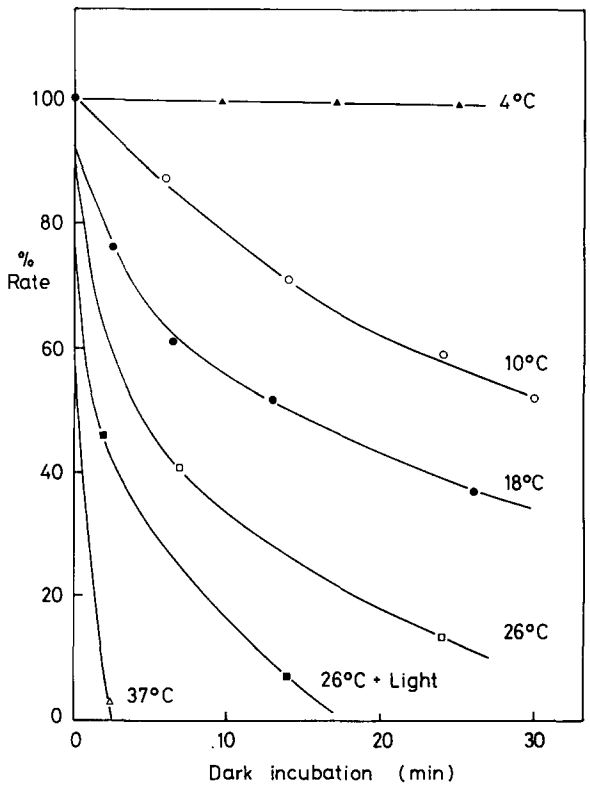

Fig. 1. Effect of temperature pretreatments on loss of relative rates of light-induced electron-transfer activity in reaction centre preparations incubated in 50 mM Tris (pH 8.0) and the dark. In one case shown the temperature pretreatment was given in the light (500 $\mu E \cdot m^{-2} \cdot s^{-1}$). The absorbance increase at 600 nm was monitored at 4°C with reaction centre samples (2 μg chlorophyll, not transferred to laurylmaltoside) in 1 ml 50 mM Tris (pH 8.0), 1 mM $MnCl_2$ and 0.2 mg silicomolybdic acid.

already reached from proteolytic digestion studies [16]. To date we have been unable to replace silicomolybdate with any other classical artificial electron acceptor, such as ferricyanide or DCIP. However, under certain conditions the addition of a quinone seems to catalyse an electron-transfer process which partially inhibits the reduction of silicomolybdate. We have found that when the Triton X-100 associated with the isolated reaction centre is exchanged with lauryl maltoside in 50 mM Tris (pH 8.0), a slow light-induced increase in absorbance is consistently observed at 430 nm when decylplastoquinone is added (see Fig. 2). Decylplastoquinone is much more hydrophilic than the naturally occurring plastoquinone-9, as judged by reverse phase high-pressure liquid chro-

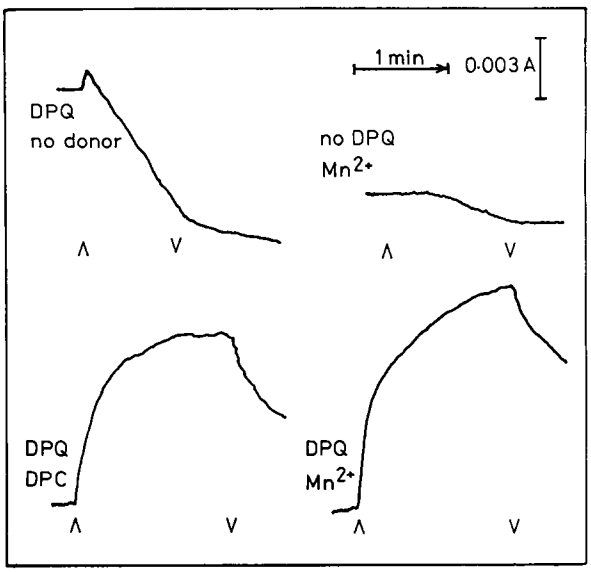

Fig. 2. Light-induced absorbance changes at 430 nm measured by split-beam spectrophotometry with illuminated and reference (dark) cuvettes containing reaction centre samples (2 μg chlorophyll, transferred to laurylmaltoside), 1 ml 50 mM decylplastoquinone (DPQ), 1 mM DPC or 1 mM $MnCl_2$ as indicated. Light on (∧), light off (∨).

matography. As shown in Fig. 2 the absorption increase at 430 nm is relatively small until electron donors, such as $MnCl_2$ or DPC are added. In this case the signal reaches a maximum and slowly reverses in the dark. Unlike the assay with silicomolybdate as an acceptor, the rate of increase at 430 nm was faster with $MnCl_2$ present than with DPC (see Table I). This effect was traced to a partial inhibition of this signal by dimethylsulphoxide which was used to dissolve DPC. Without donors the small increase in the signal is

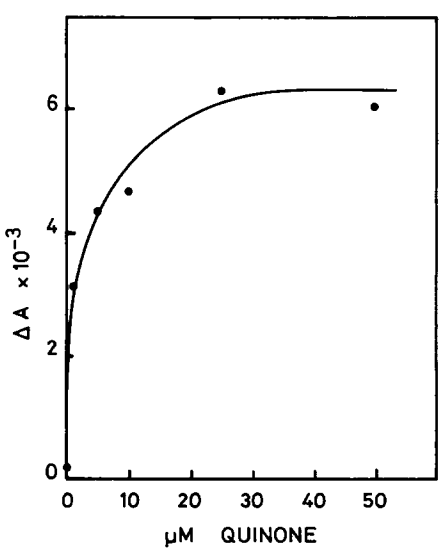

Fig. 3. Effect of decylplastoquinone concentration on the absorbance increase (430 nm), recorded after 20 s illumination of reaction centre samples (transferred to lauryl maltoside) as in Fig. 2 with 1 mM $MnCl_2$ present.

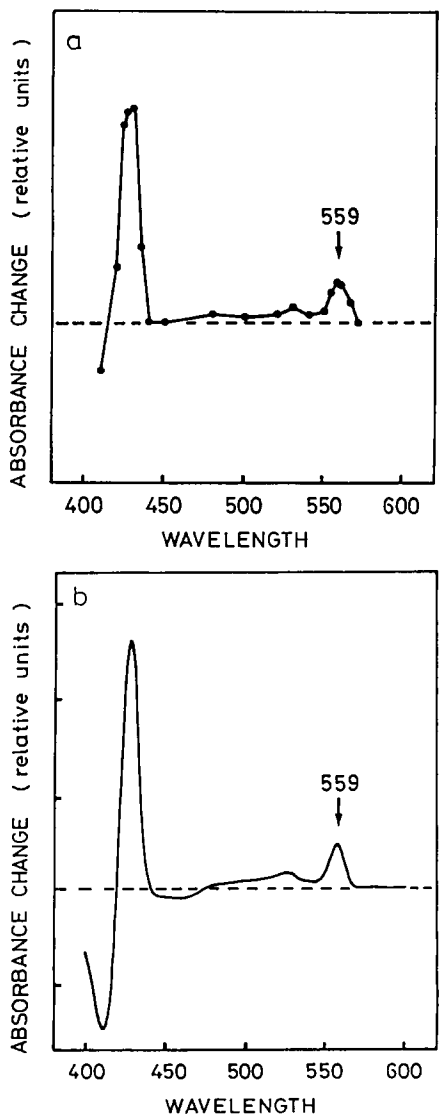

Fig. 4. Absorbance difference spectra of reaction centre preparations (transferred to laurylmaltoside). (a) Light-minus-dark difference spectrum drawn from measurement of separate samples of a preparation, with 1 mM $MnCl_2$ and 0.05 mM decylplastoquinone present as in Fig. 2. (b) Reduced (dithionite)-minus-oxidised (ferricyanide) difference spectrum.

not maintained due to a rapid steady decline in the absorbance which is also dependent on illumination. The concentration of quinone required for maximum effect is given in Fig. 3 and it should be noted that there was no positive signal at 430 nm when the decylplastoquinone was absent. The light-dark difference spectrum shown in Fig. 4 suggests that the optical-density change observed when donor and decylplastoquinone are present is due to the reduction of cytochrome *b*-559 as judged by its comparison with the reduced (dithionite) minus oxidised (ferricyanide) difference spectrum for this cytochrome measured in the isolated reaction centre (Fig. 4b). Both spectra have absorption maxima at 429, 530 and 559 nm as do spectra published for isolated cytochrome *b*-559 [19,23]. However, there is a significant difference in the Soret to alpha peak ratio of absorbances with about 5.2 for both spectra of Photosystem II reaction centre preparations and around 8.5 [19,23] for the isolated cytochrome. An explanation for the difference in the spectra is probably the comparison of the detergent derived multi-peptide complex with a purified protein which has been subjected to solvent extraction. The ability of decylplastoquinone to catalyse the light-induced reduction of cytochrome *b*-559 was inhibited when the isolated reaction centre was pre-incubated at temperatures above 4°C in a way which directly parallels the inhibition of silicomolybdate reduction (see Fig. 5). It was noted, however, that in the presence of maltoside the complex was more stable than in Triton X-100 (compare Figs. 1 and 5). Nevertheless, similar light-induced changes dependent on the presence of decylplastoquinone could also be observed without carrying out the detergent exchange but the signal sizes were variable.

A possible explanation for the above results is that the added quinone can in some way accept electrons from the reaction centre and pass them to cytochrome *b*-559. This could involve interaction with the vacant Q_A or Q_B sites which, by analogy with the purple bacterial reaction centre, should reside on the D1/D2 heterodimer. Alternatively, the added quinone may perturb the conformation of the isolated complex in some other way and allow electron transfer to the cytochrome. To check these two possibilities DCMU was added to the reaction medium. From binding studies it has already been shown that this herbicide will associate stoichiometrically with the iso-

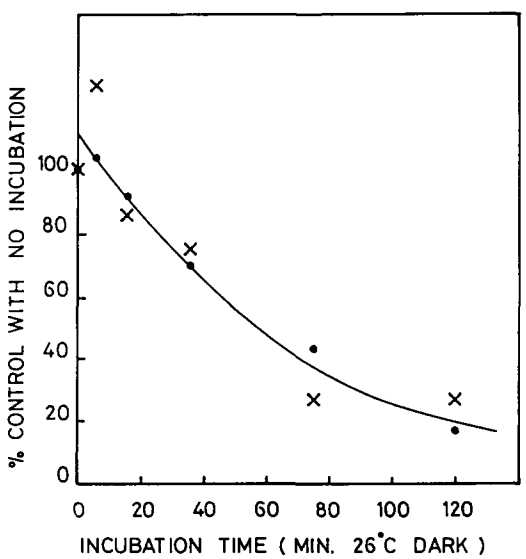

Fig. 5. Inhibition of light-dependent silicomolybdate reduction and 430 nm absorbance increase in the presence of decylplastoquinone by incubation at 26°C in the dark. A reaction centre sample (transferred to laurylmaltoside) was incubated in 50 mM Tris (pH 8.0) and aliquots taken for assay of silicomolybdate reduction (●) as in Fig. 1 and 430 nm absorbance increase (×) as in Fig. 2, with 0.05 mM decylplastoquinone and 1 mM $MnCl_2$.

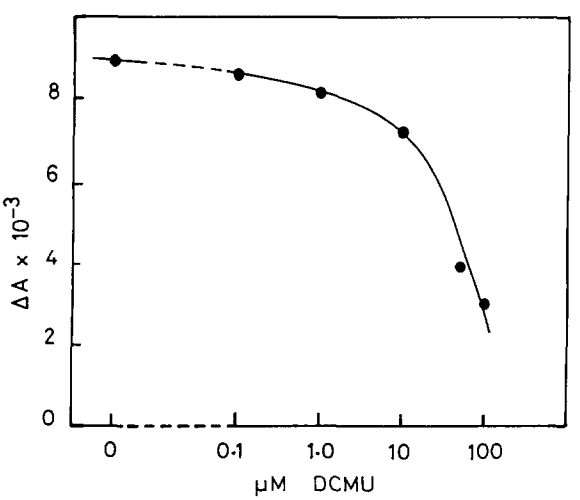

Fig. 6. Inhibition of light-induced absorbance increase of 430 nm by DCMU. Assays were as in Fig. 2 with 0.05 mM decylplastoquinone and addition of DCMU in 10 μl ethanol, using reaction centres transferred to lauryl maltoside.

lated D1/D2/cytochrome *b*-559 complex [28]. This binding, however, has a low affinity ($K_m \approx$ 12.6 μM) when compared with intact thylakoid membranes ($K_m \approx 34$ nM). Fig. 6 shows that DCMU does significantly block the decylplastoquinone catalysed light-driven reduction of cytochrome *b*-559 and, as with electron transfer in a PS II core preparation [3], a higher concentration than usual is required when compared with that needed to inhibit the PS II activity of thylakoids or membrane fragments. The determination of the redox properties of cytochrome *b*-559 with different reducing agents given in Fig. 7 shows that for the particular conditions used for the assay virtually all the cytochrome is present in its low-potential form being reducible by dithionite but not by hydroquinone. However, on addition of decylplastoquinone a significant proportion (34 ± 4%) becomes reducible by sodium borohydride. Very little ascorbate-reducible form was detected in the reaction centres even on addition of quinone (Fig. 7), a result which differs from that obtained with PS II cores by Satoh et al. [24]. However, the amount of ascorbate reducible cytochrome *b*-559 will vary with the assay system, particularly the pH. In our case the measurements in Fig. 7 were done at pH 8.0, which is the optimal pH for photoreduction of the cytochrome in the presence of decylplastoquinone.

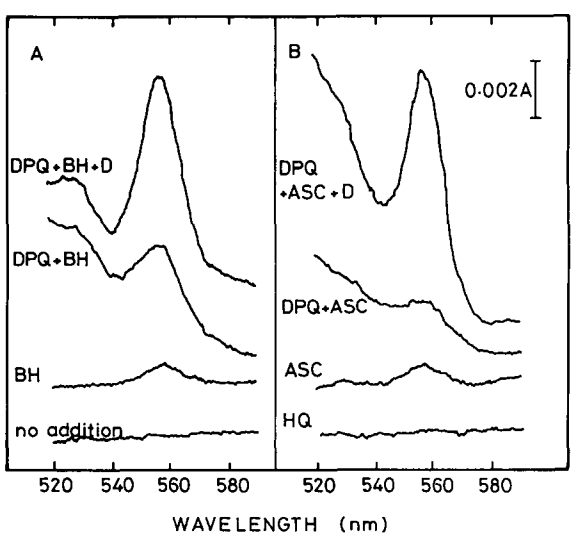

Fig. 7. Reduced-minus-oxidised difference spectra of cytochrome *b*-559. Reaction centre samples (2 μg chlorophyll in 1 ml, transferred to laurylmaltoside) were subjected to the combinations indicated where the symbols mean: 10 mM hydroquinone (HQ), 100 mM sodium ascorbate (ASC), 2 mM sodium borohydride (BH), 10 mM sodium dithionite (D) and 0.05 mM decylplastoquinone (DPQ).

Discussion

Several lines of evidence now exist which suggest that silicomolybdate can act as a legitimate secondary electron acceptor for assaying the activity of the isolated PS II reaction centre. It has already been shown that in the presence of this acceptor, but in the absence of an artificial donor, an optical absorption signal indicative of photoaccumulation of P-680$^+$ is observed with a concomitant quenching of chlorophyll fluorescence [16]. Both signals are lost when the isolated preparation is incubated at temperatures above 4° C. They are also lost or reduced significantly when DPC is added to the suspension medium [16]. In this case the preparation is still active and a net photoreduction of silicomolybdate occurs. This rate of photoreduction is sensitive to the condition of the isolated reaction centre complex, being inhibited by proteolytic treatments and by exposure to SDS [16]. In this communication we have shown that DPC can be replaced by other PS II electron donors, and in particular we have favoured the use of $MnCl_2$. Although this compound gives lower maximum rates than DPC, it does not catalyse a dark reduction of silicomolybdate and is more comparable with the natural Mn cluster of the water electron donor system. As with DPC, the Mn catalysed light induced reduction of silicomolybdate is inhibited by degradation of the PS II reaction centre complex due to light and dark incubations at temperatures above 4° C. This inhibition parallels the inhibition of decylplastoquinone catalysed light-induced reduction of cytochrome *b*-559 which perhaps can be considered a legitimate physiological assay. Therefore taken as a whole it can be concluded that the DPC or $MnCl_2$ to silicomolybdate electron-transport reaction is a true measure of the activity of the isolated PS II reaction centre consisting only of D1, D2 and cytochrome *b*-559 polypeptides.

In the absence of artificial donors or acceptors the isolated complex forms the radical pair

$P\text{-}680^+Pheo^-$ which back reacts with a half time of about 36 ns, although a small portion de-excites via a longer lived triplet state [9,10]. Presumably silicomolybdate can compete for electrons from the reduced pheophytin. In so doing some of the $P\text{-}680^+$ can be reduced by the added donor. In the absence of silicomolybdate, DPC or $MnCl_2$ are unable to compete with the back reaction and only excess dithionite is able to reduce $P\text{-}680^+$ so as to allow the photoaccumulation of reduced pheophytin [15,16]. Thus the net rate of electron flow seems to be partially governed by the ability of silicomolybdate to compete with the back reaction. Limitation must also be imposed on the donor side and indeed, some compounds are less efficient than others. Presumably, silicomolybdate receives its electrons directly from reduced pheophytin, since the process is not inhibited by DCMU [16]. It is usually assumed that artificial PS II electron donors such as DPC donate to $P\text{-}680^+$ via Z [20]. If this is true then Z must be located within the D1/D2/cytochrome *b*-559 complex in line with the recent suggestion that it is a tyrosine residue on the D1 polypeptide (Refs. 25 and 26; see also Vermaas, W., personal communication).

The fact that decylplastoquinone can mediate the photoreduction of cytochrome *b*-559 within the PS II reaction centre is an interesting and important observation. The reaction is severely inhibited by DCMU, suggesting that the electron pathway involves a herbicide binding site. Normally this site is restricted to the Q_B binding pocket on the D1 polypeptide [27], but in the absence of any quinones in the isolated complex [15] DCMU binding may also occur at the Q_A site. Thus it is conceivable that the mechanism of cytochrome *b*-559 photoreduction requires the pre-reduction of the added quinone at the Q_A and/or Q_B sites. This pre-reduction, however, seems to be relatively slow and does not compete very effectively with silicomolybdate, since in the presence of quinone the maximum inhibition of the silicomolybdate reduction rate was only 25%.

According to the maximum amplitude of the light induced signal obtained, about 30% of the cytochrome is reduced. This level of reduction was not achievable in the dark on addition of either hydroquinone, sodium borohydride or sodium ascorbate. However, when decylplastoquinone and sodium borohydride were added together, the cytochrome could be reduced to about the same extent as the light-driven process. This observation suggests that decylplastoquinone is directly involved in the light-induced reduction of cytochrome *b*-559. This conclusion, together with the fact that the photoreduced cytochrome is re-oxidised in the dark, even in the presence of electron donors such as $MnCl_2$, makes this light-induced signal intriguing and worthy of further investigation.

Acknowledgements

We wish to thank the Agricultural and Food Research Council and the Science and Engineering Research Council for financial help. We acknowledge the technical help given by Kenneth Davis and John De Felice and are grateful to Dr. Peter Rich for supplying us with decylplastoquinone and for discussions regarding its role in the photoreduction of cytochrome *b*-559.

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