Biochimica et Biophysica Acta, 723 (1983) 247–255
Elsevier

BBA 41298

RECOGNITION OF INTERACTION BETWEEN THE DONOR ELECTRON-TRANSFER CHAINS OF PHOTOSYSTEM II UNDER CONDITIONS OF PARTIAL INHIBITION OF OXYGEN EVOLUTION

NIGEL K. PACKHAM and JAMES BARBER

ARC Photosynthesis Research Group, Department of Pure and Applied Biology, Imperial College of Science and Technology, London SW7 2BB (U.K.)

(Received December 3rd, 1982)

Key words: Photosynthesis; Photosystem II; Oxygen evolution; Electron transfer; Thylakoid membrane; (Pea chloroplast)

The electron-transfer pathway on the donor side of Photosystem (PS) II has been examined using unfractionated and inside-out thylakoid membrane vesicles. A number of treatments are identified which result in the inhibition of light-dependent oxygen evolution. The differential capacities of the exogenous donors diphenylcarbazide and NH_2OH to restore the PS II-mediated reduction of 2,6-dichlorophenolindophenol (DCIP) in the inhibited membranes is discussed in terms of multiple donor sites for the electron-transfer pathway on the oxidising side of PS II. We also present data which indicate that the donor chains are not isolated from each other but that an individual PS II reaction centre may be able to interact with several oxygen-evolving complexes. The implication of such an interaction to the mechanism of oxygen evolution is discussed.

Introduction

It is known that the photosynthetic generation of molecular oxygen in chloroplasts is driven by the photo-oxidised P-680 chlorophyll of PS II reaction centres [1–3]. Our present incomplete understanding of this important reaction is partly due to the inner membrane location and to the paucity of specific inhibitors of the electron-transfer reactions taking place on the donor side of PS II. The recent introduction of procedures for isolating inverted thylakoid membranes [4,5] has enabled new experimental approaches to be adopted. Indeed, studies have revealed new treatments which irreversibly block oxygen evolution (e.g., monovalent cations [5], EDTA [6] and KCN [7]) only when applied to the inverted thylakoid membranes.

Much of our detailed knowledge of the reactions giving rise to oxygen evolution is derived from kinetic measurements. The elegant studies of Joliot and Kok and colleagues [8–10] led to the concept of the S-state cycle of water oxidation in which each oxygen-evolving complex is associated with a specific reaction centre. Although this model has been widely accepted, the interpretation of the damping of the flash-induced O_2 yields in terms of 'double hits' and 'misses' has been questioned [11–14]. For example, the high quantum yields observed for photosynthetic reaction centres [15] are difficult to reconcile with the proposal that the 'misses' are due either to a rapid back-reaction

Abbreviations: PS, photosystem; P-680, the reaction centre chlorophyll of PS II; Z, the uncharacterised immediate donor to P-680; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; EGTA, ethylene bis(oxyethylenenitrilo)tetraacetic acid; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; Mes, 2-(*N*-morpholino)ethanesulphonic acid; Tricine, *N*-tris(hydroxymethyl)-methylglycine.

within a reaction centre, or to the existence of transiently blocked, and thereby, photo-unreceptive reaction centres.

In this paper we give evidence that exogenous electron donors can differ in their ability to restore activity in inhibited PS II centres, which indicate that there could be multiple donor sites in the electron-transport pathway between the oxygen-evolving complex and P-680. Our data also suggest that a component in this electron-transport chain is able to interact with several PS II reaction centres. Such an interaction may account for the damping in the observed oscillations of the flash-induced oxygen yield experiments and gives support for the mathematical analysis by Lavorel [16,17] of the mechanism of damping.

Materials and Methods

Unfractionated thylakoid membranes were isolated from *Pisum sativum* (Feltham First) using standard procedures and were routinely suspended in a high-salt buffer medium containing 150 mM NaCl, 50 mM phosphate buffer, pH 7.4. Chlorophyll concentrations were determined in 80% acetone [18]. Inside-out and right-side-out thylakoid vesicles were prepared from the unfractionated membranes by passage through a Yeda Press and by phase separation as described by Andersson et al. [4]. The various inhibitory treatments on the membranes were performed as follows:

KCN treatment. This was performed as described in detail in Ref. 7. The inside-out thylakoid membranes were incubated in 100 mM phosphate buffer, pH 7.5, and 10 mM KCN for 15 min under low light.

EDTA treatment. The inside-out vesicles were washed with 2 mM EDTA in 0.1 M sucrose, pH 7.5, for 30 min on ice, at a concentration of approx. 200 μg Chl/ml [6], and control inside-out vesicles were incubated in the presence of 10 mM NaCl. The vesicles were recovered, before assay of PS II activity, by centrifugation at $35\,000 \times g$ for 30 minutes.

Trypsin digestion. Thylakoid membranes, suspended at a concentration of approx. 250 μg Chl/ml were incubated with 50 μg trypsin (Sigma Chemical Co., Poole, U.K.) for 15 min at room temperature (18°C). The reaction was stopped by the addition of 250 μg trypsin inhibitor (Sigma Chemical Co.) and the vesicles were recovered by centrifugation.

Tris inactivation. Unfractionated membranes (equivalent to 1 mg Chl) were treated with 40 ml of high-salt phosphate buffer supplemented with 50 mM Tricine (pH 8.3) at 4°C. The amount of Tris present was varied between and 0 and 0.8 M. After 15 min incubation at 4°C, the membranes were recovered by centrifugation.

pH inactivation. Unfractionated membranes (equivalent to 1 mg Chl) were suspended in 40 ml of 150 mM NaCl, containing 10 mM each of Hepes, Tricine and glycylglycine at a pH which was varied between 7.5 and 9.2. The membranes were recovered by centrifugation after a 15 min incubation at room temperature.

The PS II mediated electron-transfer reactions were assayed as follows:

Oxygen evolution. Oygen evolution was measured by polargraphy (Rank Brothers Ltd., Bottisham, Cambridge) in a medium containing 0.3 M sorbitol, 100 mM phosphate buffer, pH 7.5, and 10 mM NaCl. Exciting light was provided by an Intralux 150 H light source, filtered through a red-transmitting Corning Filter. 100% light intensity was approx. 10 $J \cdot s^{-1} \cdot m^{-2}$ and, where appropriate, the intensity was reduced using neutral density filters (Balzer Ltd.).

DCIP reduction. DCIP reduction was monitored spectrophotometrically [19] using a Perkin-Elmer (Model 557) dual-wavelength spectrophotometer. The cuvette routinely contained 1 ml of high-salt buffer and membranes corresponding to a chlorophyll concentration of between 5 and 40 μg with 0.1 mM DCIP. Actinic light was provided by an Intralux 150 H light source fitted with a 668 nm interference filter (Balzer B.40). The 100% light intensity was about 10 $J \cdot s^{-1} \cdot m^{-2}$, and where appropriate the intensity was attenuated by Balzer neutral density filters.

All exogenous electron donors were prepared just prior to use; NH_2OH was prepared as a 0.5 M stock solution dissolved in high-salt buffer to pH 7.5 and diphenylcarbazide was prepared as a 50 mM stock solution in DMSO. DCMU was dissolved in methanol. In all the experiments where NH_2OH was added, the concentrations used were sufficient to inactivate fully the oxygen evolution

rate. Computed rates of DCIP reduction were corrected for any dark reaction rate.

Results

The different efficacies of NH_2OH and diphenylcarbazide in restoring PS II-mediated electron-transfer reactions after the inhibition of oxygen evolution

We have previously shown that, under certain well defined ionic conditions, photosynthetic oxygen evolution in inverted thylakoid vesicles can be inhibited by KCN and EDTA [6,7]. Both agents specifically affect inside-out thylakoid vesicles, indicating that the exposure of an inner membrane surface component(s) is necessary for the expression of inhibition. In an attempt to identify the location of the site of action we have compared the effectiveness of the exogenous electron donors NH_2OH and diphenylcarbazide in restoring PS II-mediated reactions.

Fig. 1A shows that the DCMU-sensitive light-dependent reduction of DCIP, supported by water oxidation, is slowed by the pretreatment of the inside-out thylakoid vesicles with 2 mM EDTA. The addition of diphenylcarbazide to the inverted membranes resulted in an increased rate of DCIP

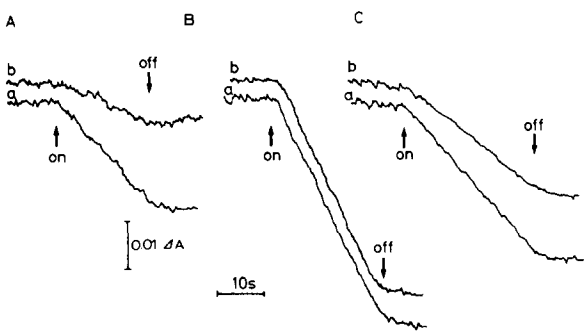

Fig. 1. EDTA-induced inhibition of the light-dependent DCIP reduction supported either by H_2O or NH_2OH oxidation. The conditions for the experiments including the EDTA inhibition are given in Materials and Methods. The chlorophyll concentration was 7 μg/ml. Uncoupled rates of DCIP reduction for the untreated membranes (a) and EDTA-washed membranes (b) are indicated by the slope of absorption decrease. The various donor conditions were: (A) H_2O to DCIP; (B) diphenylcarbazide (0.5 mM) to DCIP; (C) NH_2OH (5 mM) to DCIP. The maximal rate of DCIP reduction (e.g., as in condition B) was 112 μmol DCIP/mg Chl per h.

reduction which was unaffected by the EDTA pretreatment, indicating that EDTA induces a block on the oxidising side and not the acceptor side of PS II. A stimulation by diphenylcarbazide over the water oxidation rate is not detected in unfractionated membranes (data not shown) which suggests that the inside-out thylakoid vesicles have a reduced water oxidation capacity; a conclusion strengthened by the observations of decreased oxygen evolution rates from this type of fractionated membrane [7]. We have also examined the effect of NH_2OH in supporting DCIP reduction. Unlike diphenylcarbazide, NH_2OH is able to inhibit water oxidation [1–3], and the observed decreased rate of NH_2OH donation in EDTA-washed membranes (Fig. 1C) indicates that EDTA can inhibit both water and NH_2OH oxidation rates. A slowed DCIP reduction rate supported either by water or NH_2OH oxidation has also been detected in unfractionated membranes treated with 2 mM EGTA (data not shown, see Ref. 20), and this presumably is due to the ability of the chelator, unlike EDTA, to traverse the thylakoid membrane.

A similar distinction between the capacities of diphenylcarbazide and NH_2OH to donate reducing equivalents to inhibited PS II reaction centres was detected in inside-out thylakoid vesicles treated either with KCN (Fig. 2A and B) or by mild trypsin digestion (Table I). As we observed for the EDTA inhibition, diphenylcarbazide was the more effective donor in restoring the light-dependent reduction of DCIP after both treatments had inhibited water oxidation. In Fig. 2B it is clear that a slow rate of DCIP reduction could nevertheless be maintained by NH_2OH, and this may reflect the incomplete extent of cyanide inhibition.

The results of all three inhibitory treatments of inside-out thylakoid vesicles suggest that diphenylcarbazide and NH_2OH have different sites to which they can donate reducing equivalents to the PS II reaction centre. It would appear that diphenylcarbazide donates close to the P-680 chlorophyll of the PS II reaction cente while NH_2OH donates closer to the site of water oxidation. To investigate this further we have examined the action of alkaline Tris on the electron-transfer efficacies of the two exogenous donors

In Fig. 3 we show the results of a series of

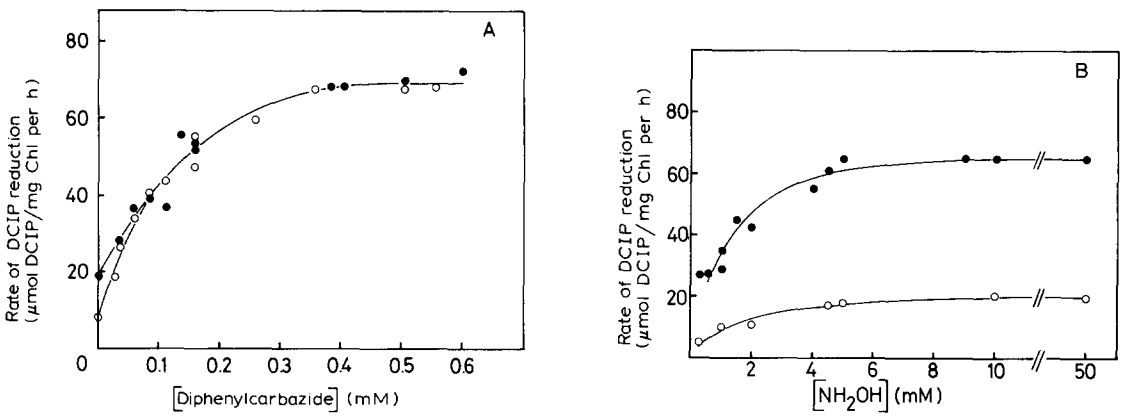

Fig. 2. Differential efficacies of diphenylcarbazide and NH_2OH in restoring the light-dependent DCIP reduction in cyanide-treated thylakoid membranes. The assay of DCIP reduction using the various exogenous electron donors to PS II was undertaken as described for Fig. 1. Cyanide inhibition was obtained by the pre-illumination of the inside-out thylakoid vesicles in the presence of 10 mM KCN, as described in Ref. 7. PS II activity was subsequently assayed in samples containing 25 μg Chl/ml and at a final cyanide concentration of 0.5 mM. (A) Effect of diphenylcarbazide on the rate of DCIP reduction; (B) Efficacy of NH_2OH in supporting DCIP reduction. (●———●) Control, (○———○) + 10 mM KCN.

experiments in which the H_2O to DCIP electron-transfer rate was inhibited to varying extents up to about 90% by increasing the severity of the alkaline Tris treatment [21–23]. As can be seen, the same treatments had little effect on the diphenyl-

TABLE I

COMPARISON OF THE ABILITY OF DIPHENYLCARBAZIDE AND NH_2OH TO REACTIVATE PS II-SUPPORTED ELECTRON TRANSFER IN TRYPSIN-DIGESTED THYLAKOID MEMBRANES

Assay conditions as for Fig. 1. Reduction rates expressed as μmol DCIP/mg Chl per h.

Membrane fraction	$H_2O \rightarrow$ DCIP	$NH_2OH \rightarrow$ DCIP	Diphenyl carbazide/ $NH_2OH \rightarrow$ DCIP
Inside-out thylakoids:			
(a) Untreated	56	70	126
(b) Trypsin-treated	0	4	53
Right-side-out thylakoids			
(a) Untreated	120	72	124
(b) Trypsin-treated	36	21	56

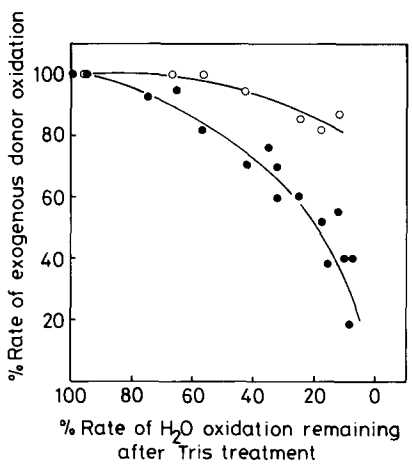

Fig. 3. Inactivation of water oxidation by alkaline Tris: effect of exogenous donor oxidation. Unfractionated thylakoid membranes were treated with Tris at pH 8.3 as described in Materials and Methods. The degree of inhibition caused by Tris treatment was determined from the attenuation of the rate of DCIP reduction supported by water oxidation. Assay conditions as for Fig. 1, except for a chlorophyll concentration of 50 μg/ml. For each inhibited sample the rate of DCIP reduction supported by diphenylcarbazide (0.5 mM) and NH_2OH (5 mM) was measured and plotted against the DCIP reduction rate supported by H_2O oxidation. 100% control values were: 132 and 122 μmol DCIP/mg Chl per h for the H_2O and NH_2OH oxidation rates, respectively. Addition of diphenylcarbazide to the control sample did not stimulate the rate of DCIP reduction. (●———●) NH_2OH oxidation, (○———○) diphenylcarbazide oxidation.

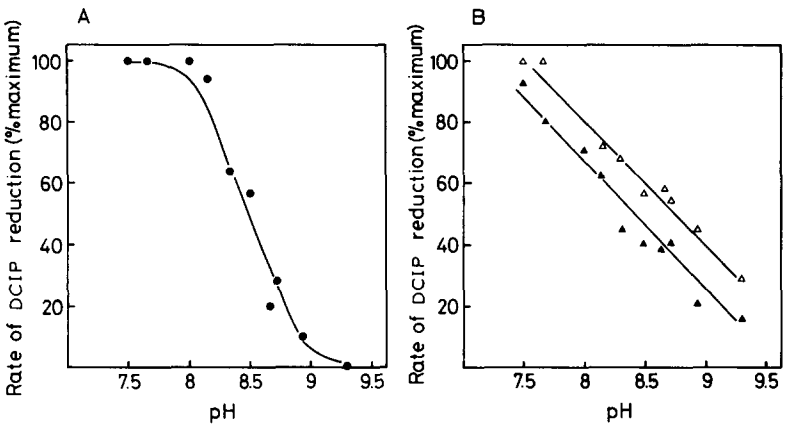

Fig. 4. Inhibition of PS II activity by alkaline pH. Unfractionated thylakoid membranes were treated with alkaline pH as described in Materials and Methods. The assay of DCIP reduction was undertaken at pH 7.5 as described for Fig. 3. (▲———▲) NH_2OH oxidation, (△———△) diphenylcarbazide oxidation.

carbazide to DCIP electron-transfer rate but inhibited the NH_2OH-supported reduction of DCIP. It should be noted, however, that with mild treatments of Tris the extent of inhibition of the NH_2OH to DCIP electron-transport rate does not correlate with the significant loss of the water oxidation, suggesting that the artificial electron donor system has a different sensitivity to Tris inactivation. Similar results have been obtained using inverted thylakoid membranes.

The Tris inactivation experiments were performed at a constant pH of 8.3, close to the pK for the Tris buffer. It has been reported that unfractionated thylakoid membranes incubated at high pH in the absence of Tris also show decreased rates of water oxidation [24,25] and this result is

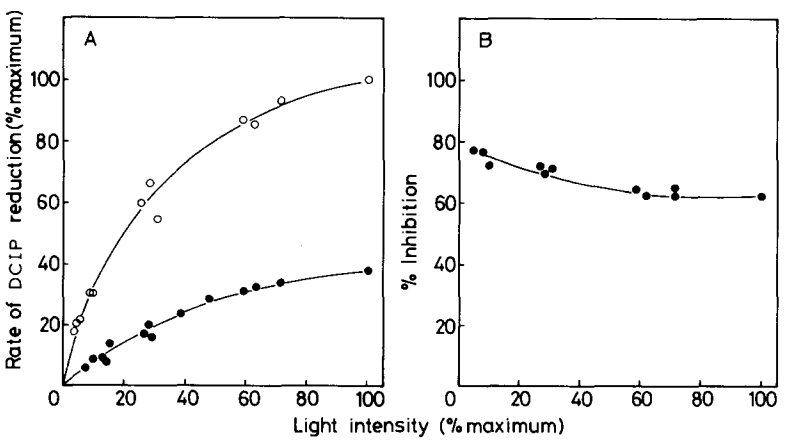

Fig. 5. Comparison of the DCIP reduction rates supported by H_2O or diphenylcarbazide oxidation in inside-out thylakoid vesicles: effect of light intensity. Assay conditions as for Fig. 1. The light intensity was attenuated by neutral density filters. 100% actinic light intensity determined to be 10 $J \cdot s^{-1} \cdot m^{-2}$. (A) Light intensity saturation curves for H_2O to DCIP (●———●) and diphenylcarbazide to DCIP (○———○) electron transfer. 100% rate determined to be 95 μmol DCIP/mg Chl per h. (B) Plot of the apparent extent of inhibition of the H_2O-supported DCIP reduction rate. The extent of inhibition was computed from the subtraction of the light intensity curve for H_2O oxidation from the curve obtained for diphenylcarbazide oxidation, and expressed as a percentage. The points represent the manipulation of the data, indicating the degree of error.

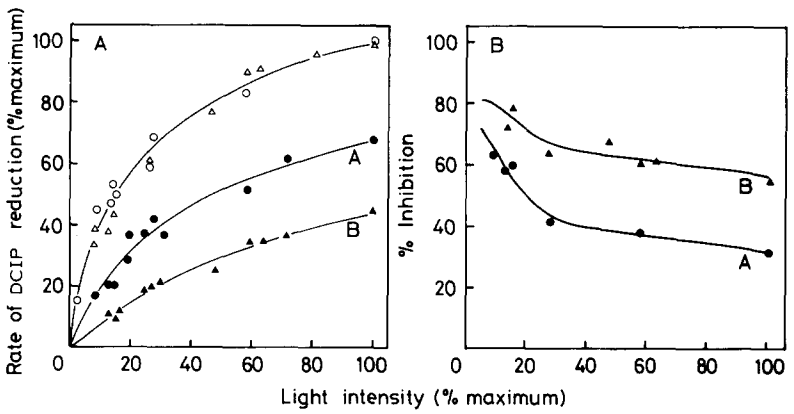

Fig. 6. The effect of EDTA on the light intensity saturation curves. Assay of DCIP reduction obtained following the procedure described in Fig. 1. (A) Comparison of the rates of H_2O to DCIP electron transfer in untreated thylakoid membranes (curve A) and in EDTA-washed membranes (curve B). The open circles (○——○) and triangles (△——△) refer to the rates of DCIP reduction with the addition of diphenylcabazide in the control and EDTA-washed membranes, respectively. (B) plot of the apparent extent of inhibition of the H_2O to DCIP electron-transfer rates, compared to the diphenylcarbazide to DCIP rates, in control membranes (curve A) and EDTA-treated membranes (curve B).

shown in Fig. 4A for the H_2O to DCIP reaction. When the ability of NH_2OH and diphenylcarbazide to restore the light-dependent DCIP reduction was examined, we noted a similar pH-dependent inhibition for both donors (see Fig. 4B). This result contrasts with the differential action of the two donors observed with the other inhibitory treatments. It is worth noting, however, that the pH sensitivity curves for the artificial donors and for water oxidation differ in that the latter does not follow a classical Henderson-Hasselbach relationship for a weak base.

The differential ability of H_2O and diphenylcarbazide to reduce DCIP at varying light intensities

We have routinely observed that the rate of light-induced oxygen evolution from inside-out thylakoid vesicles is lower (typical values exhibit a

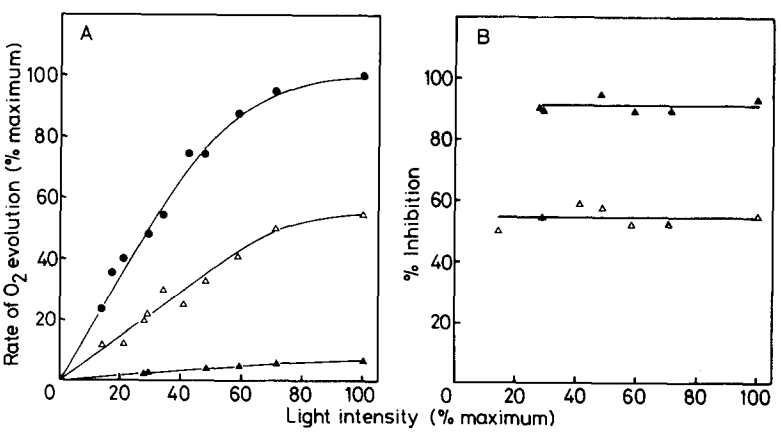

Fig. 7. The effect of DCMU of the light intensity saturation curves. Assay of the light-dependent oxygen evolution was undertaken as described in Materials and Methods. Unfractionated membranes (50 μg Chl/ml) were suspended in the high-salt buffer with 0.1 mM benzoquinone as the electron acceptor and 5 mM NH_4Cl as uncoupler. DCMU added as a methanol solution. (A) Comparison of the rates of oxygen evolution is untreated and DCMU-inhibited membranes. 100% rate equivalent to 94 μmol O_2/mg Chl per h. (B) Plot of the extent of inhibition of oxygen evolution caused by DCMU at varying light intensities. (●——●) Control, (△——△) 10^{-7} M DCMU, (▲——▲) $5 \cdot 10^{-7}$ M DCMU.

30–50% attenuation) than the corresponding rate with unfractionated membranes. This inhibition is most probably introduced during the Yeda press fractionation of the thylakoid membrane, and since the addition of diphenylcarbazide restores fully the light-induced rate of DCIP reduction we can assume that only the donor pathway of PS II is affected. The relative efficiency of H_2O and diphenylcarbazide to donate to PS II reaction centres of inverted thylakoid membranes was examined at differing light intensities. In Fig. 5A it can seen that at high light intensities diphenylcarbazide causes a stimulation in the DCIP reduction rate above that due to water oxidation. The extent of the stimulation in rate was found to vary with the light intensity; H_2O becomes a less effective donor at lower light intensities. This result is expressed in Fig. 5B where the ratio of the two rates at different light intensities has been plotted. Parenthetically, in a study of the rate of DCIP reduction in unfractionated membranes we could not detect an affect of diphenylcarbazide on the normal uninhibited rate due to water oxidation at any light intensity. This result indicates that for fully functional unfractionated membranes the quantum efficiency of H_2O oxidation when compared to that of diphenylcarbazide oxidation is not varying with light intensity. Therefore, the result of Fig. 5 indicates that with inside-out thylakoid vesicles the donor electron-transfer chains have been altered so as to reduce the efficiency of water oxidation, relative to diphenylcarbazide oxidation, and this inhibition is more pronounced at the lower light intensities.

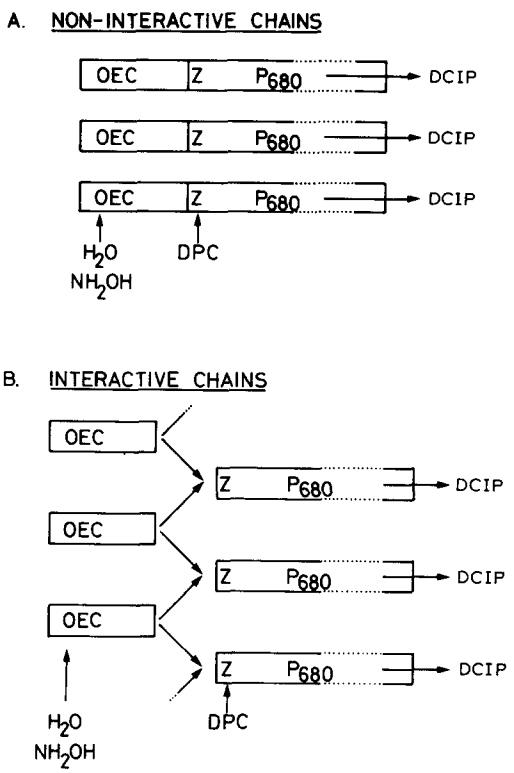

Fig. 8. Model of the donor electron-transport pathway of PS II. The oxygen-evolving complexes (OEC) and the PS II reaction centres (containing Z and the P-680 chlorophyll) are envisaged either as being spatially inseparate (A, non-interactive chains) or spatially distinct (B, interactive chains). It is presumed that diphenylcarbazide is able to feed electrons directly to the intemediary carrier Z, whereas H_2O and NH_2OH donate electrons somewhere on the OEC itself. See text for further details. DPC, diphenylcarbazide.

In Fig. 6 we show that the apparent extent of inhibition of H_2O oxidation, relative to diphenylcarbazide oxidation, caused by EDTA treatment of inside-out thylakoid vesicles also increases at the lower actinic light intensities. We have observed similar results with unfractionated membranes in which the water oxidation rate was partially inhibited by treatment with either EGTA or Tris (data not shown). In contrast to these results obtained with agents which affect the donor electron transfer of PS II, the extent of inhibition of the acceptor reactions of PS II caused by the addition of DCMU was observed to be constant over a wide range of light intensities (Fig. 7).

Discussion

The results presented in this paper suggest that specific regions of the donor electron-transport pathway of PS II can be probed by a comparison of the effect of the exogenous donors NH_2OH and diphenylcarbazide. We have observed that several treatments of inverted thylakoid membranes which cause the inhibition of H_2O oxidation (e.g., EDTA, KCN and trypsin) will also affect the ability of NH_2OH to restore the PS II-mediated reduction of DCIP. On the other hand, these same treatments had little or no effect on the diphenylcarbazide to DCIP electron-transfer process. Thus, it can be concluded that diphenylcarbazide is able

to feed electrons either to a component of the electron-transfer chain between oxygen-evolving complex and P-680 which is closer to the reaction centre than the site of NH_2OH donation, or that diphenylcarbazide by-passes the normal chain and donates by an alternative pathway to P-680. The former possibility is more likely because earlier work has indicated that diphenylcarbazide can reduce the light-oxidised intermediate Z, as shown by an increased relaxation rate of this EPR-detectable species in Tris-washed chloroplasts [26]. The site of electron donation by NH_2OH is unknown, but could be at the oxygen-evolving complex itself, since the donor is isoelectronic with water [27]. However, the action of low concentrations of Tris indicates that the NH_2OH electron donation is more resistant to inhibition by this compound than the H_2O oxidation process (see Fig. 3). Our observation that the efficacy of NH_2OH to donate electrons to PS II is lost at severest Tris treatments agrees with the earlier finding [28] that NH_2OH donation in manganese-depleted membranes varies with the extent of manganese deficiency. Our observations that both NH_2OH and diphenylcarbazide fail to restore DCIP reduction at high pH suggests that alkaline conditions impair electron flow close to the PS II reaction centre and beyond that of the diphenylcarbazide donation site; a result which is consistent with the finding that Z oxidation is impeded at pH values above 8.0 [29].

The light intensity curves of DCIP reduction supported by either H_2O or diphenylcarbazide have yielded results which can be interpreted as indicating a degree of interaction between the electron-transfer chains on the donor side of PS II. At low light intensities, the H_2O-supported electron flow was apparently less efficient than the diphenylcarbazide to DCIP rate. This effect was detected with either unfractionated membranes with O_2 evolution rates partially inhibited by treatment with EGTA or Tris, or with inside-out thylakoid vesicles which possess a reduced capacity to oxidise water relative to rates of diphenylcarbazide oxidation (see Figs. 5 and 6). No change in the efficiency of water oxidation could be detected, however, when the number of active PS II centres was partially reduced by DCMU (Fig. 7). It therefore seems that the observed phenomenon is due to the partial inhibition of that region of the H_2O to P-680 chain which does not support diphenylcarbazide oxidation. Such a result would be unexpected for non-interactive electron-transfer chains (see Fig. 8A), since the difference between the H_2O and diphenylcarbazide-supported reduction rates of DCIP would be anticipated to be constant as the light intensity is lowered. If there is a possibility, however, of an interaction between electron-transfer chains on the donor side of PS II which involves a diffusional step (of the type shown in Fig. 8B), then the observed changes between the H_2O to DCIP and diphenylcarbazide to DCIP rates at varying light intensities can be explained. At all intensities, diphenylcarbazide oxidation can maintain a light-dependent maximum rate of electron flow through its more direct access to the photo-oxidised P-680. At high light intensities the partial inhibition of the electron-transfer rate for H_2O to DCIP will reflect the degree of inhibition of the donor chains close to the oxygen-evolving complexes. At low light intensities, where the photochemical turnover of the reaction centres is limited, the proposed diffusional step will introduce a further attenuation in the electron-transfer rate. This is due to the increased relative spatial separation between the photooxidised reaction centres and the functionally active oxygen-evolving complexes. Such a model assumes that individual PS II reaction centres can interact with more than one oxygen-evolving complex.

The idea of a diffusion-controlled interaction between the donor chains of PS II is not new, and has been implied in the theoretical work of Lavorel [16,17] and more recently by protein isolation studies using inside-out thylakoid vesicles [5,6]. This latter work indicates the existence of extrinsic proteins on the inner surface of the thylakoid membrane, which could be envisaged as mobile electron carriers between spatially distinct oxygen-evolving complexes and PS II reaction centres. The postulated interaction is not readily detected when diphenylcarbazide does not stimulate the rate of DCPIP reduction above that of water oxidation, and where all the oxygen-evolving complexes seem to be fully functional. Thus, for a fully functional system the rate of water oxidation is not limited by the postulated diffusional process.

At first sight it may appear that the concepts of

interaction between donor electron-transfer chains of PS II is inconsistent with the tenets of the Kok S-state hypothesis of oxygen evolution. But if we accept the assumption that individual oxygen-evolving complexes require four oxidising equivalents before releasing oxygen, then our proposal of a limited interaction between donor chains of PS II may provide a mechanism to account for the damping of the flash-induced oxygen yields. The damping will arise from the statistical distribution of photo-oxidised PS II centres associated with an oxygen-evolving complex at any given time. In those PS II centres with no oxygen-evolving complex closely associated, electron flow from water oxidation is slowed and may compete unfavorable against a back-reaction from the reduced acceptors. The net result for these decoupled centres is an apparent photochemical miss and a retardation of the S-state transition. From the work presented in this paper, it is expected that conditions which evoke a partial inhibition of the oxygen-evolving complexes will affect the degree of damping in the oxygen yield. In a numerical analysis of a similar interactive model, Lavorel [17] has shown that the damping in oxygen yield upon repetitive flashes is influenced by both the degree of interaction between donor chains and the flash frequency.

The results presented in this paper suggest that the path of electrons from the site of water oxidation to the P-680 reaction centre chlorophyll of PS II may involve the interaction of more than one protein complex. We might expect that an alteration in the spatial separation between the complexes, by the manipulation of the parameters which govern protein-protein and protein-lipid interactions will have pronounced effects on the water oxidation rate especially at low light intensities.

Acknowledgements

We wish to thank the Nuffield Foundation, the Science and Engineering Research Council and the Agricultural Research Council for financial support. We also acknowledge Kathy Wilson for technical assistance and Roy Mansfield for helpful discussions.

References

1 Radmer, R. and Cheniae, G. (1977) in Primary Processes of Photosynthesis (Barber, J., ed.), pp. 303–348, Elsevier, Amsterdam
2 Bouges-Bocquet, B. (1980) Biochim. Biophys. Acta 594, 85–103
3 Velthuys, B.R. (1980) Annu. Rev. Plant Physiol. 31, 545–567
4 Andersson, B. and Akerlund, H.E. (1978) Biochim. Biophys. Acta 503, 462–472
5 Akerlund, H.E., Jansson, C. and Andersson, B. (1982) Biochim. Biophys. Acta 681, 1–10
6 Mansfield, R.W. and Barber, J. (1982) Biochem. Biophys. Res. Commun. 110, 545–551
7 Packham, N.K., Mansfield, R.W. and Barber, J. (1982) Biochim. Biophys. Acta 681, 538–541
8 Joliot, P., Barbieri, G. and Chabaud, R. (1969) Photochem. Photobiol. 10, 309–329
9 Kok, B,, Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475
10 Joliot, P. and Kok, B. (1975) in Biochemistry of Photosynthesis (Godvindjee, ed.), pp. 387–413, Academic Press, New York
11 Delrieu, M.J. (1980) Biochim. Biophys. Acta 592, 478–494
12 Lavorel, J. (1976) J. Theor. Biol. 57, 171–185
13 Thibault, P. (1978) J. Theor. Biol. 73, 271–284
14 Beckwith, A.C. and Jursinic, P.A. (1982) J. Theor. Biol. 97, 251–265
15 Wraight, C. and Clayton, R.K. (1974) Biochim. Biophys. Acta 333, 246–260
16 Lavorel, J. (1976) FEBS Lett. 66, 164–167
17 Lavorel, J. (1982) in From Cyclotrons to Cytochromes. Essays in Molecular Biology and Chemistry (Kaplan, N.O. and Robinson, A., eds.), pp. 323–345, Academic Press, New York
18 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
19 Vernon, L.P. and Shaw, E.R. (1969) Plant Physiol. 44, 1645–1649
20 Barr, R., Troxel, K.S. and Crane, F.L. (1980) Biochem. Biophys. Res. Commun. 92, 206–212
21 Yamashita, T. and Butler, W.L. (1968) Plant Physiol. 43, 1978–1986
22 Cheniae, G.M. and Martin, I.F. (1978) Biochim. Biophys. Acta 502, 321–344
23 Yamamoto, Y., Dai, M., Tamura, N. and Nishimura, M. (1981) FEBS Lett. 132, 265–268
24 Horth, E., Reimer, S. and Trebst, A. (1974) FEBS Lett. 42, 165–168
25 Maison-Peteri, B., Vernotte, C. and Briantais, J.M. (1981) Biochim. Biophys. Acta 637, 202–208
26 Babcock, G.T. and Sauer, K. (1975) Biochim. Biophys. Acta 396, 48–62
27 Bouges-Bocquet, B. (1973) Biochim. Biophys. Acta 292, 772–785
28 Heath, R.L. and Hind, G. (1969) Biochim. Biophys. Acta 189, 222–233
29 Bowes, J.M., Crofts, A.R. and Itoh, S. (1979) Biochim. Biophys. Acta 547, 336–346