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Mode of Action Studies on Nitrodiphenyl Ether Herbicides[1]

II. The Role of Photosynthetic Electron Transport in *Scenedesmus obliquus*

John R. Bowyer*, Beverly J. Hallahan, Patrick Camilleri, and Joy Howard

Department of Biochemistry, Royal Holloway and Bedford New College, Egham Hill, Egham, Surrey, TW20 0EX, United Kingdom (J.R.B., B.J.H., J.H.), *and Shell Research Ltd., Sittingbourne Research Centre, Sittingbourne, Kent, ME9 8AG, United Kingdom* (P.C.)

ABSTRACT

The nitrodiphenyl ether herbicide 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitroacetophenone oxime-*o*-(acetic acid, methyl ester) (DPEI) induces light- and O_2-dependent lipid peroxidation and chlorophyll (Chl) bleaching in the green alga *Scenedesmus obliquus*. Under conditions of O_2-limitation, these effects are diminished by prometyne and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), both inhibitors of photosynthetic electron transport. Mutants in which photosynthetic electron transport is blocked are also resistant to DPEI under conditions of O_2-limitation. Light- and O_2-dependent lipid peroxidation and Chl bleaching are also induced by 5-[2-chloro-4-(trifluoromethyl)phenoxy]-3-methoxyphthalide (DPEII), a diphenyl ether whose redox properties preclude reduction by photosystem I. However, these effects of DPEII are also inhibited by DCMU. Under conditions of high aeration, DCMU does not protect *Scenedesmus* cells from Chl bleaching induced by DPEI, but does protect against paraquat. DPEI, but not paraquat, induces tetrapyrrole formation in treated cells in the dark. This is also observed in a mutant lacking photosystem I but is suppressed under conditions likely to lead to O_2 limitation. Our results indicate that, in contrast to paraquat, the role of photosynthetic electron transport in diphenyl ether toxicity in *Scenedesmus* is not to reduce the herbicide to a radical species which initiates lipid peroxidation. Its role is probably to maintain a sufficiently high O_2 concentration, through water-splitting, in the algal suspension.

The *p*-nitrodiphenyl ether herbicides, such as 2-chloro-1-(3-ethoxy-4-nitrophenoxy)benzene (oxyfluorfen), cause rapid light- and O_2-dependent peroxidation of unsaturated membrane lipids and bleaching of Chl and carotenoid in leaves (14, 17, 23). These effects are similar to those of 1,1′-dimethyl-4,4′-bipyridinium dichloride (paraquat). Paraquat and other bipyridinium herbicides are reduced by PSI and reoxidized by O_2, generating superoxide and, ultimately, hydrogen peroxide and hydroxyl radicals, which initiate lipid peroxidation (reviewed in Summers [26]). This led to the suggestion that the initial event in DPE[2] toxicity was reduction of the DPE to a radical anion by PSI (15). In the case of the *p*-nitro-DPEs, however, photosynthetic electron transport appears to play a more ambiguous role. Thus, a number of photosynthetically incompetent plant materials (barley plants lacking either PSI or PSII (4), cucumber seedlings grown under far red light (8), a nonchlorophyllous soybean cell suspension (21, 22) and tomato cell suspension (11), are very susceptible to DPEs, but, at least in some cases, have been shown to be resistant to paraquat. In photosynthetically active tissue, inhibition of photosynthetic electron transport with DCMU markedly inhibits paraquat toxicity, as expected (4), but has variable effects on DPE action. In the green alga *Scenedesmus obliquus*, for example, DCMU completely protects cells against lipid peroxidation and Chl bleaching induced by the DPE oxyfluorfen (15). This observation was taken as evidence supporting the hypothesis that the initial event in DPE toxicity was its reduction to a radical by PSI. However, while Matringe *et al.* (20) reported that DCMU protected greened cucumber seedlings from LS 82-556, a compound with apparently the same mode of action as DPEs, other workers have reported little or no protection against DPEs by DCMU in photosynthetically competent tissue (4, 8, 9), when protection against paraquat is observed (4, 9). In addition, the fact that certain compounds have very similar toxic effects to DPEs on plants, but possess redox properties which would preclude their reduction by PSI (9, 10) suggests that other explanations must be sought to account for the involvement of photosynthetic electron transport in the mode of action of DPEs.

In order to eliminate any possibility that the results of Kunert and Böger (15) on *Scenedesmus* reflected a specific secondary effect of DCMU unrelated to inhibition of photosynthetic electron transfer, we have extended their observations by using a different electron transport inhibitor (prometryne) and a different DPE, (DPEI), and also by using mutants of *Scenedesmus* which lack parts of the photosynthetic electron transport chain. In the light of the recent results of Matringe and Scalla (22), and see also Halling and Peters

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[2] Abbreviations: DPE, diphenyl ether; LS82-556, (*S*)3-*N*-(methylbenzyl)carbamoyl-5-propionyl-2,6-lutidine; DPEI, 5-[2-chloro-4(trifluoromethyl)phenoxyl]-2-nitroacetophenone oxime-*o*-(acetic acid, methyl ester); E_7[1], one-electron reduction potential at pH 7 *versus* normal hydrogen electrode; DPE II, 5-[2-chloro-4-(trifluoromethyl)phenoxy]-3-methoxyphthalide.

(12), showing that acifluorfen-methyl induces abnormal tetrapyrrole accumulation which could then photosensitize singlet O_2 formation leading to cellular damage, we have also examined the ability of DPEI to induce tetrapyrrole formation in *Scenedesmus* under various conditions.

Our results support the conclusion that a functioning photosynthetic electron transport chain is required to elicit DPE-toxicity in *Scenedesmus* under certain conditions but that its role is secondary and acts by maintaining a sufficiently high O_2 concentration though water photolysis, rather than to reduce the herbicide to a radical state.

MATERIALS AND METHODS

Algal Culture

Scenedesmus obliquus wild-type strain D3 and photosynthetic mutants were obtained from Prof. N. I. Bishop, Oregon State University. The mutants lacked parts of the electron transport chain (PS-CPa_I-11 lacking PSI activity because it lacks P700, Chl-protein I [CP-a_I] and two iron-sulfur proteins; PS-b_6f-2 which lacks the Cyt b_6f complex; and PS-X-C_{14}-11 lacking PSII activity because it is deficient in CP-a_{II}1 and CP-a_{II}-2 and their respective apoproteins of M_r 48 kD and 44 kD, as well as the three extrinsic proteins involved in the water-splitting complex [N.I. Bishop, personal communication]). Cultures were maintained in darkness on an enriched agar medium (modified Kessler's medium [3] plus 0.5% w/v glucose and 0.25% w/v yeast extract [2]). Cells in suspension were grown heterotrophically on the same medium at 22°C at a light intensity of 0.27 Wm^{-2} (measured over the range 450–900 nm) provided by white fluorescent lights. All cells were grown heterotrophically because the nonphotosynthetic mutants are unable to grow autotrophically. Pigment-protein complexes and photosynthetic electron transport chains (incomplete in the mutants) are produced under these conditions [2, 3]. A low light intensity was used to ensure photoactivation of the water-splitting complex of PSII when present (7), but minimizing photo-damage to the nonphotosynthetic mutants. Cells were grown in 75 mL of medium in 250 mL Erlenmeyer flasks on an orbital shaker rotating at 150 rpm. They were subcultured (200 μL inoculum) every 4 d and each experiment was initiated 4 d after the previous transfer. Cells were harvested by centrifugation and resuspended in fresh sterile growth medium to a concentration of 23 mg wet weight per mL. Wet weights were estimated by vacuum filtering a known volume of cell suspension through a premoistened Whatman GF/F filter. Cells were lyophilized for dry weight determinations.

Hydrocarbon Formation in Herbicide-Treated Algae

For measurements of ethane formation arising from lipid peroxidation, 5 mL of concentrated cell suspension were placed in a 10 mL Erlenmeyer flask and the required herbicide added. The flask was then sealed with a Subaseal No. 33 rubber seal and flushed for 10 min using a hydrocarbon-free synthetic air mixture (80% N_2, 20% O_2, 0.03% CO_2). The flasks were placed on an orbital shaker rotating at 150 rpm at 20 ± 2°C under a light intensity of 330 Wm^{-2} (measured over the range 450–900 nm) at the surface of the algal suspension. Illumination was provided by an array of 150 W floodlights. Sample heating was avoided using a perspex trough through which water circulated, placed between the floodlights and the sample. At intervals, 0.5 mL samples of head space were removed from the flasks using a gas tight syringe, replaced with air, and analyzed by gas chromatography using a Pye Unicam 204 Gas Chromatograph. The column (152 cm long, 0.4 cm internal diameter) was filled with activated alumina (80–100 mesh). The injector and detector were maintained at 150°C and the column at 110°C. The column was calibrated using 1 μL/L ethane in N_2 (Argo International). Ethane production was calculated from GC peak heights, and a value for the total volume of the head space in each flask.

Chl Bleaching in Herbicide-Treated Algae

For experiments in which Chl bleaching was used to monitor DPE toxicity, 5 mL samples of concentrated algal suspension in 10 mL conical flasks were set up on an orbital incubator at 25°C but the flasks were either stoppered with a gas-tight Subaseal, or with cotton wool, permitting aeration during the incubation with the herbicide. Illumination for these experiments was provided by an array of 60 W bulbs (20 cm apart and 22 cm above the flasks) providing an intensity at the surface of the algal suspension of 190 ± 10 Wm^{-2} (at 450–900 nm). At intervals, 0.5 mL samples of suspension were removed using a hypodermic syringe under sterile conditions, centrifuged, and pigments were extracted from the algal pellet by heating for 6 min in 96% ethanol at 80°C (28). The Chl *a* content of the extract was estimated spectrophotometrically by measuring at $A_{665\ nm}$ and $A_{649\ nm}$ and using the equation of Lichtenthaler and Wellburn (19).

Measurements of One-Electron Reduction Potentials

The one-electron reduction potentials of DPEs were measured from the equilibrium concentrations of the reduced and oxidized forms after pulse radiolysis (29) in the presence of 1,1′-butano-2,2′-bipyridinium dibromide. Deoxygenated solutions in propan-2-ol/5 mM aqueous phosphate buffer (pH 7) (1:3 v/v) containing 10 to 40 μM DPE and 30 to 90 μM 1,1′-butano-2,2′-bipyridinium dibromide (E_7^1 −635 mV in aqueous phosphate buffer [1]) were irradiated with electron pulses as described by Wardman and Clarke (29). Rather low concentrations of redox compounds were used in these measurements owing to the low solubilities of the DPEs.

Tetrapyrrole Accumulation in Herbicide-Treated Algae

For these experiments, cells were grown as described earlier either under low light (0.27 Wm^{-2}) or high light (200 Wm^{-2}) for several generations. Four d old cultures were harvested and resuspended to 30 mg wet weight per mL in 5 mL of fresh medium, and treated with herbicides as described earlier. Flasks were stoppered with cotton wool or Subaseals and incubated in the dark at 25°C on an orbital shaker for 24 h. Tetrapyrroles were extracted using a modification of the method of Rebeiz *et al.* (25). The cell suspension was centrifuged at 3,000*g* for 5 min and the pellet resuspended in 1 mL

0.1 M NH_4OH. This suspension was added dropwise to liquid nitrogen in a mortar and the cells ground to a fine powder. The powder was suspended in 9 mL of acetone and centrifuged at 3,000*g* for 5 min. The supernatant was removed and the extraction repeated twice. The acetone extracts were combined and centrifuged at 39,000*g* for 10 min at 4°C. The supernatant was washed with 2 equal volumes of hexane. The final volume of the acetone extract was 10 mL. Fluorescence emission spectra of the hexane-extracted acetone extracts were obtained using a Perkin Elmer 3000 fluorescence spectrometer, with excitation and emission slit widths of 10 nm, and an excitation wavelength of 398 nm.

Other Methods

Rates of O_2 uptake or evolution by cell suspensions were measured at 20°C using a Clark type O_2 electrode (Rank Brothers, Cambridge) and illumination was provided by a 150 W floodlight filtered by a Schott RG 610 filter and 10 cm water giving an intensity greater than 200 Wm^{-2}.

With the exception of paraquat, herbicide stock solutions were made up in DMSO giving 0.1% v/v DMSO after dilution into the algal suspensions. Controls contained 0.1% v/v DMSO. Paraquat was obtained from Sigma Chemical Co. and the other herbicides were kind gifts from agrochemical companies, as listed in "Acknowledgments."

All results presented are the means from two replicates of at least two separate experiments, except where indicated.

RESULTS

The results in Figure 1, A and B, show that DPEI caused similar levels of ethane formation in wild-type illuminated cells of *Scenedesmus* as oxyfluorfen, which was used in earlier studies by Kunert *et al.* (15, 16, 18). However, while 10 μM DPEI was more effective than 1 μM DPEI, in the case of oxyfluorfen, 10 μM appeared to be less effective than 1 μM. In addition, 1 μM DPEI completely inhibited the heterotrophic growth of *Scenedesmus* (Fig. 2) in complete darkness (as opposed to the low light intensity normally used for growth). The same observation was made using oxyfluorfen (not shown). As has been demonstrated with oxyfluorfen (15, 16, 18), concurrent treatment of the cells with DCMU almost completely inhibited the action of both DPEI and paraquat in wild type cells (Fig. 3, A and B). No ethane formation was induced by DPEI in the absence of illumination (not shown). Ethane formation by DPEI was also inhibited by the PSII electron transport inhibitor prometryne as effectively as DCMU (not shown). Figure 3A shows that very little ethane formation was induced by either paraquat or DPEI in a mutant of *Scenedesmus* lacking PSI. Similar results were obtained using mutants lacking PSII or the Cyt b_6f complex (not shown).

Neither oxyfluorfen nor DPEI markedly inhibited photosynthetic electron transport or respiratory oxygen uptake in cells on a minutes time scale (*e.g.* 10 μM oxyfluorfen inhibited CO_2-dependent oxygen evolution by 10% within 2 min of its addition, but no further inhibition was observed after 10 min [data not shown]).

The effects of a novel phthalide DPE, DPEII, are illustrated in Figure 4. DPEII has effects on a range of plants which are indistinguishable from those of herbicidal nitrodiphenyl ethers (rapid wilting, necrosis, and chlorosis [5]). Pulse radiolysis studies of DPEII in propan-2-ol/water (1:3 v/v) indicate that reduced 1,1′-butano-2,2′-bipyridinium dibromide, E_7^1 −635 mV) was unable to reduce DPEII, indicating a one-electron reduction potential for DPEII of less than −700 mV, whereas that for DPEI was −563 mV. DPEII induced marked ethane formation in *Scenedesmus* in a light-dependent process and concurrent treatment with DCMU provided complete protection (Fig. 4).

We next attempted to demonstrate that O_2 was necessary for the lipid peroxidation induced by DPEI and DPEII in *Scenedesmus*. To study the effect of O_2 availability on ethane formation, algal suspensions were sealed into 10 mL conical

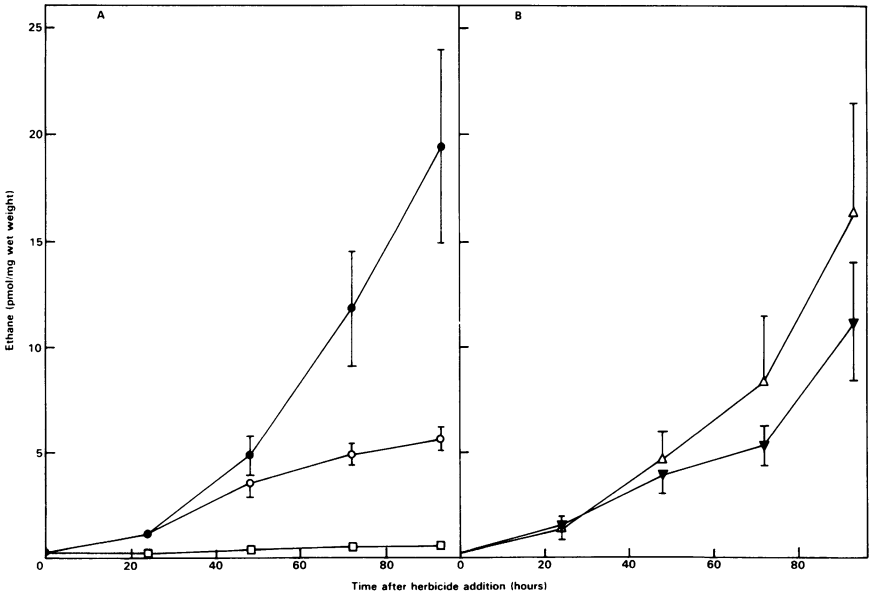

Figure 1. Effect of DPE herbicides on lipid peroxidation in illuminated *Scenedesmus* cells as measured by monitoring ethane formation. A, Effect of oxyfluorfen at 1 μM (●) and 10 μM (○); control (□). B, Effect of DPEI at 1 μM (▼) and 10 μM (△). The error bars where shown indicate the SE based on seven measurements.

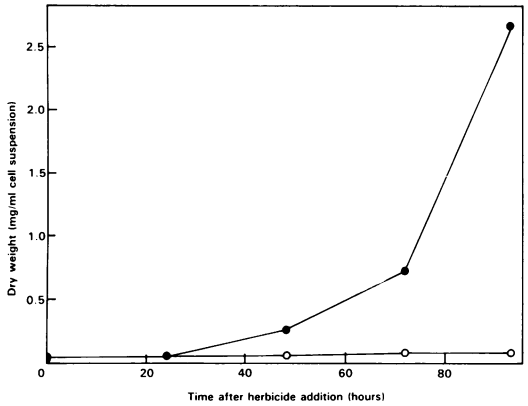

Figure 2. Effect of DPEI on the heterotrophic growth of a *Scenedesmus* mutant lacking PSI in complete darkness. DPEI was added to 1 μM to a culture which had just been inoculated (○); control (●). Similar results were obtained for wild type and mutants lacking PSII or the Cyt b_6f complex.

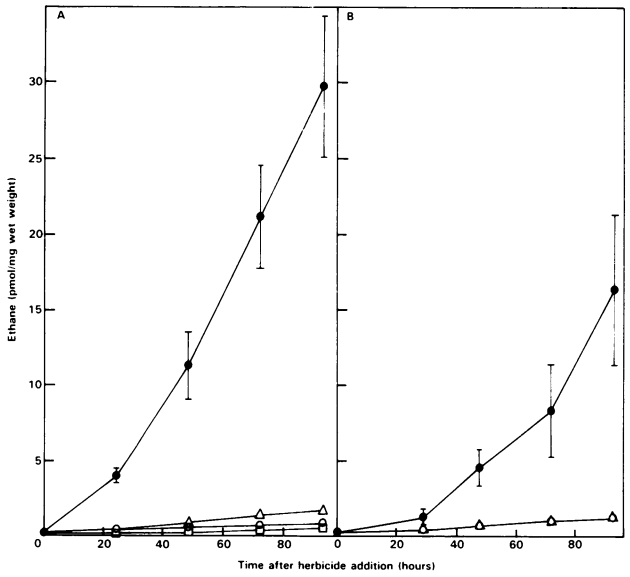

Figure 3. Effect of inhibition of photosynthetic electron transport on lipid peroxidation induced by DPEs in illuminated *Scenedesmus*, monitored using ethane formation. A, Effect of 2 mM paraquat on wild type (●); effect of 2 mM paraquat on mutant lacking PSI (△); effect of 2 mM paraquat and 10 μM DCMU on wild type (○); and control, PSI mutant (□). B, Effect of 10 μM DPEI on wild type (●); effect of 10 μM DPEI on mutant lacking PSI (△); and, superimposed, effect of 10 μM DPEI and 10 μM DCMU on wild type (○). The error bars where shown indicate the SE based on seven measurements.

flasks with Subaseals, and the flasks were then flushed either with compressed air or with N_2 for 10 min. The results in Figure 4 show that with either DPEI or DPEII, the flasks flushed with air showed greater rates of ethane formation than those flushed with N_2.

To demonstrate a requirement for O_2 in the Chl bleaching induced by DPEI, we compared the extent of bleaching in cultures sealed with Subaseals with that in cultures in flasks lightly stoppered with cotton wool and shaken at a high speed on the orbital shaker (200 rpm) in order to achieve high aeration. The results in Figure 5 show that after a lag period

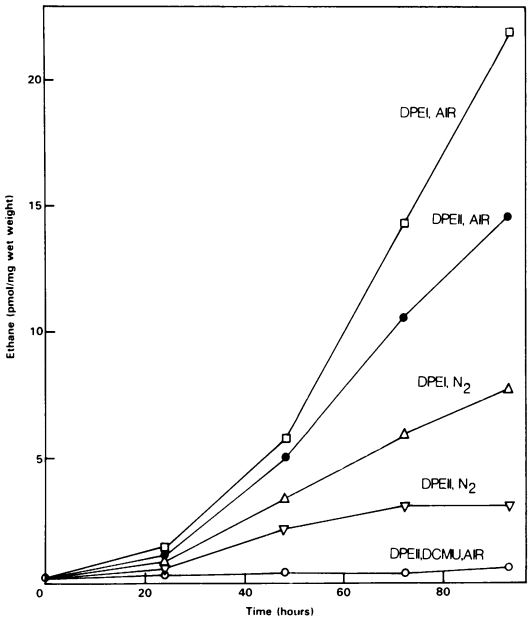

Figure 4. Effect of O_2 on the ability of DPEs to induce lipid peroxidation monitored by ethane formation in illuminated *Scenedesmus* cells. At the beginning of the experiment, flasks were flushed with either air or N_2 for 10 min. The additions were 10 μM DPEI with air (□), 10 μM DPEII with air (●); 10 μM DPEI with N_2 (△); 10 μM DPEII with N_2 (▽); 10 μM DPEII with 10 μM DCMU and air (○); 10 μM DPEII with air, in darkness, superimposed on (○); and 10 μM DCMU with air, superimposed on (○).

of about 30 h, net Chl bleaching is induced by both DPEI and paraquat in the vigorously aerated culture. In the Subasealed flasks, no Chl bleaching was observed, although DPEI did partially inhibit Chl accumulation (result not shown). Similarly in Subasealed flasks flushed daily with nitrogen, no net Chl bleaching was induced by DPEI or paraquat (not shown). These results indicate that more vigorous aeration is needed to elicit Chl bleaching than lipid peroxidation, since DPEI did elicit ethane formation in the Subasealed flasks. The results in Figure 5 also show that in vigorously aerated suspensions, DCMU inhibited Chl bleaching in cells treated with paraquat, but not with DPEI. However, in Subasealed flasks vigorously aerated for 10 min after the gas space had been sampled at each time point, we were unable to overcome the inhibition by DCMU of DPEI-induced ethane formation (not shown).

The fluorescence emission spectra (Fig. 6) of hexane-washed acetone extracts of *Scenedesmus* show that cells incubated with DPEI in darkness accumulate a pigment (Fig. 6B) which had the same spectral characteristics as protoporphyrin IX (Fig. 6A), *i.e.* an emission maximum on our spectrofluorimeter of 628 nm and an excitation maximum (not shown) of 398 nm. In the algal extracts, the 628 nm emission is seen as a shoulder on a much larger emission band peaking at 670 nm, which is attributed to more polar Chl derivatives not extracted by the hexane washing. DPEI did not have consistent effects on this component. The accumulation of tetrapyrrole in the dark in dark grown *Scenedesmus* treated with DPEI was only seen in flasks lightly stoppered with cotton wool, but not in Subasealed flasks (compare Fig.

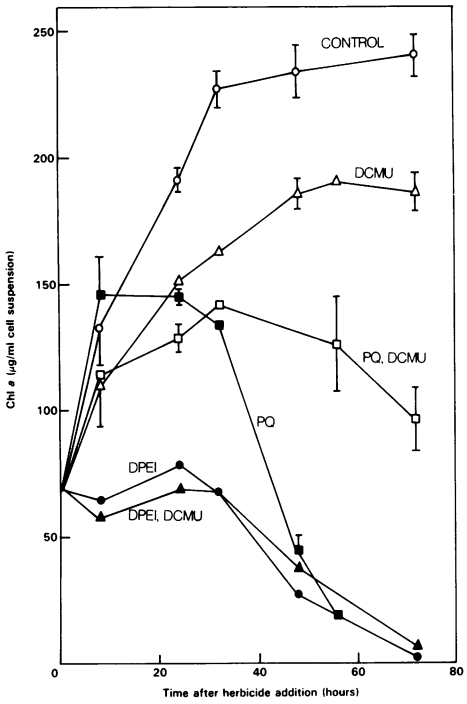

Figure 5. Effect of aeration on the ability of DCMU to protect illuminated *Scenedesmus* cells from paraquat and DPEI, as measured using bleaching of Chl *a*. Cells were initially suspended to 0.012 g/mL wet weight in growth medium and dispersed into 10 mL Erlenmeyer flasks which were then lightly stoppered with cotton wool. They were placed on an orbital shaker rotating at 200 rpm to achieve high aeration. Effects of 10 μM DPEI (●), 10 μM DCMU (△), 10 μM DPEI with 10 μM DCMU (▲); 2 mM paraquat (■), and 2 mM paraquat with 10 μM DCMU (□); control (○). The error bars where shown indicate the SE based on at least four measurements.

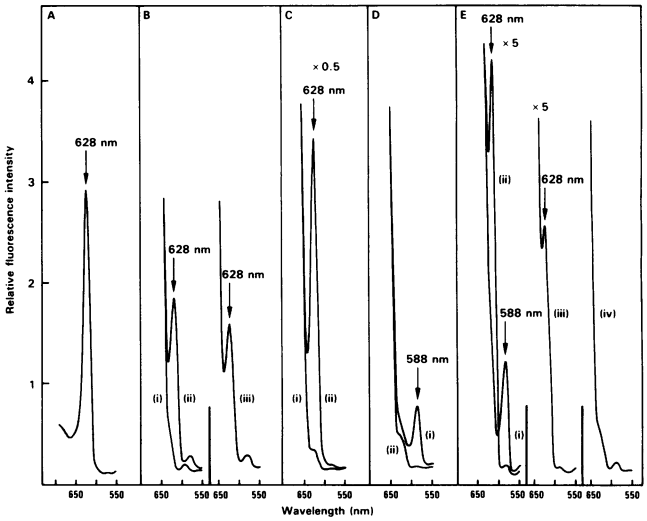

Figure 6. Fluorescence emission spectra of acetone extracts of *Scenedesmus* cells incubated with 0.1% (v/v) DMSO (i), 10 μM DPEI (ii), 10 μM DCMU + 10 μM DPEI (iii), and 2 mM paraquat (iv) for 24 h in the dark. Spectra were elicited by excitation at 398 nm. A, Protoporphyrin IX in 9:1 (v/v) acetone:0.1 M NH_4OH. B, Wild-type cells, grown under low light intensity (0.27 Wm^{-2}), incubated in flasks stoppered with cotton wool. C, Mutant cells lacking PSI grown under low light intensity, incubated in flasks stoppered with cotton wool. D, Wild-type cells, grown under low light intensity, incubated in flasks stoppered with Subaseals. E, Wild-type cells grown under high light intensity (200 Wm^{-2}), incubated in flasks stoppered with Subaseals.

6, B and D). In the Subasealed flasks, an unidentified component with an emission maximum at 588 nm appeared, which was suppressed by DPEI. DCMU slightly suppressed DPEI-induced tetrapyrrole accumulation (Fig. 6, B and E). This could not be related to inhibition of photosynthetic electron transport since the incubations were carried out in darkness. Furthermore, tetrapyrrole accumulation was also induced by DPEI in the mutant lacking PSI (Fig. 6C). In contrast to dark-grown cells, light-grown cells incubated in darkness with DPEI in Subasealed flasks did show tetrapyrrole accumulation, but to a considerably lower extent than that in dark grown cells lightly stoppered with cotton wool. Paraquat did not induce tetrapyrrole accumulation (Fig. 6E).

DISCUSSION

DPEI induces light- and O_2-dependent lipid peroxidation in *Scenedesmus* by a process which is suppressed by the photosynthetic electron transport inhibitors DCMU and prometyne. Curiously, 1 μM oxyfluorfen was more effective than 10 μM oxyfluorfen, an effect not noted by Kunert and Böger (15), nor seen with DPEI. It may result from a more rapid decline in the rate of photosynthetic electron transport and O_2 evolution with 10 μM oxyfluorfen than with 1 μM (*e.g.* see Fig. 1C in Ref. 15), resulting in the same effect as DCMU addition. The mechanism by which oxyfluorfen and DPEI inhibit dark heterotrophic growth of *Scenedesmus* is unknown. However, *Scenedesmus*, unlike higher plants, synthesises Chl and assembles a complete photosynthetic apparatus in darkness. Thus if growth is obligatorily coupled to this process, then DPEs would inhibit growth if the induced tetrapyrrole accumulation results from an inhibition of Chl biosynthesis. This is consistent with the observation that LS82-556 does not inhibit the dark heterotrophic growth of a nonchlorophyllous soybean cell suspension (21). It is also possible that DPEs inhibit both Chl biosynthesis and heme synthesis, which would prevent the assembly of a complete respiratory chain needed for aerobic heterotrophic growth.

The results obtained using mutants blocked in photosynthetic electron transport are consistent with the effects of DCMU and support the idea that photosynthetic electron transport has an essential role in the toxic action of DPEs on *Scenedesmus*. These results with *Scenedesmus* contrast with the effects of DCMU (4, 8, 9) or the absence of PSI or PSII (4) on DPE toxicity in other systems. In higher plants, these measurements and studies on photosynthetically incompetent cucumber seedlings (8) indicate that photosynthetic electron transport is not required to elicit DPE toxicity. However, the protective effect of DCMU in *Scenedesmus* led to the proposal that the DPE had to be reduced to a radical anion by PSI before it could initiate lipid peroxidation (15).

The pulse radiolysis studies with DPEII show that its one-electron reduction potential must be less than −700 mV. The photo-activated pigments of PSI (P^*_{700}) and PSII (P^*_{680}) and their initial acceptor chains (A_0-A_1-FeS_X for PSI and phaeo-

phytin *a* for PSII) have redox properties (midpoint potentials ranging from −1.35 V for P^*_{700} to −0.73 V for FeS_X (6, 13, 30)) which would enable them to reduce a component with a midpoint potential of −700 mV. However, it is likely that the actual midpoint potential of DPEII is very much lower than −700 mV, and even if the reduction of DPEII was thermodynamically possible, it would probably not occur at a significant rate since electron transfer from the initial acceptors in the photochemical reaction centers to less reducing components in the chains (*e.g.* FeS_A and FeS_B in PSI with midpoint potentials of −550 mV and −585 mV respectively [6]) occur very rapidly [27]. Thus, while PSI has the potential to reduce DPEI, it is highly unlikely that it can reduce DPEII. The results with DPEII therefore show that the role of photosynthetic electron transport in eliciting DPE toxicity in *Scenedesmus* is not to reduce the DPE to a radical anion. Lambert *et al.* (18) also showed that DCMU protected *Scenedesmus* from lipid peroxidation induced by methyl-5-[2-chloro-4(trifluoromethyl)phenoxy]-2-chlorobenzoate, a compound which also does not readily form free radicals (10).

The requirement for O_2 to elicit Chl bleaching and lipid peroxidation suggests a role for photosynthetic electron transport in DPE-toxicity in *Scenedesmus*. Under the conditions of our experiments, in which algae are suspended in heterotrophic growth medium, respiration will lower the O_2 concentration, particularly if photosynthetic O_2 evolution is blocked in flasks sealed with Subaseals. Thus, the respiratory rate of cells in fresh medium at the concentration used for the ethane formation studies was 100 μmol $O_2 \cdot$ mg $Chl^{-1} \cdot h^{-1}$. The total O_2 content of a flask equilibrated with air would be about 95 μmol, so that in the absence of photosynthesis, all the O_2 in the flask could be consumed by respiration in approximately 3 h, a short time period compared with that required to induce ethane accumulation. Thus photosynthetic electron transport would have the dual role of generating O_2 and reducing the herbicide in the case of paraquat toxicity, but would only have the former role in the case of DPEs. Such a hypothesis would be consistent with the different effect of blocking photosynthetic electron transport on paraquat- and DPE-induced Chl bleaching in the vigorously aerated cell suspension. In experiments with barley seedlings, DPEs did induce ethane formation in mutants unable to evolve O_2 photosynthetically (4). However, O_2-limitation is less likely in these experiments since the leaves were not submerged, and they were only sealed into small tubes to measure ethane formation for a 4 h period at each time point.

The experiments on tetrapyrrole accumulation are consistent with the results of Matringe and Scalla (22) and further support the secondary role of photosynthetic electron transport in DPE-toxicity in *Scenedesmus*. Thus, if as seems likely, DPE toxicity is linked to tetrapyrrole accumulation, then, since DPEI did elevate the amount of tetrapyrrole in the mutant lacking PSI the failure to observe ethane formation in this mutant must be a secondary consequence of the mutation. Tetrapyrrole accumulation was, however, suppressed in algal suspensions in which O_2 should have been depleted as a result of respiration. It is known that O_2 is required as the oxidant in the oxidation of protoporphyrinogen to protoporphyrin (24). The increased accumulation of tetrapyrrole in light grown versus dark grown cells in Subasealed flasks could then be attributed to the higher respiration rate of the latter, on a wet weight basis (result not shown). According to this hypothesis, O_2 is required for both the synthesis of tetrapyrrole, and as the substrate for singlet O_2 formation photosensitized by the tetrapyrrole; photosynthesis is then only required as a source of O_2 from water oxidation under conditions of O_2 limitation.

The experimental conditions employed by Kunert and Böger (15) (autotrophically growing cells bubbled with air) would be less likely to lead to O_2 limitation under conditions of inhibited photosynthesis, but they still observed an inhibition by DCMU of oxyfluorfen-induced lipid peroxidation and Chl bleaching and we also were unable to overcome the DCMU block on DPE-induced lipid peroxidation by increased aeration. However, the destruction of cytochromes induced by oxyfluorfen was only slightly diminished by DCMU, which lead Kunert *et al.* (16) to propose O_2-limitation as the cause of the DCMU effect. To overcome the DCMU block on DPE-induced lipid peroxidation it will probably be necessary to measure lipid peroxidation under conditions of continuous vigorous aeration using a sensitive and specific assay which does not depend on hydrocarbon gas accumulation.

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