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Chloroplast-Diphenyl Ether Interactions II[1]

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ABSTRACT

Acifluorfen, a *p*-nitrodiphenyl ether herbicide, is inhibitory to those photosynthetic functions that require a functioning chloroplast envelope. Functions involving the stroma are also affected. Acifluorfen does not lyse intact spinach chloroplasts, yet does increase the sensitivity of CO_2-dependent O_2 evolution to exogenous inorganic phosphate without directly affecting the function of the phosphate translocator. Acifluorfen penetrates into the chloroplast stroma in a light-independent fashion. Once inside, it causes the inactivation of light and dithiothreitol-activated fructose 1,6-bisphosphatase. Light-activated glyceraldehyde-3-phosphate dehydrogenase (NADP) is also inactivated by acifluorfen.

These data suggest that acifluorfen stimulates a pathway for inactivation of fructose 1,6-bisphosphatase and glyceraldehyde 3-phosphate dehydrogenase (NADP) which uses oxygen as a terminal oxidant and which involves thioredoxin and ferredoxin-thioredoxin reductase.

The *p*-nitrodiphenyl ethers, represented by acifluorfen (sodium 5-[2-chloro-4-(trifluormethyl)phenoxy]-2-nitrobenzoate), demonstrate an absolute light requirement for herbicidal activity. Carotenoids have been implicated as photoreceptors for DPE[2] action (5, 6, 22) although the nature of the interaction between herbicide and photoreceptor is not understood. Evidence to date suggests that the peroxidation of membrane lipids by oxygen radicals occurs as a consequence of DPE action (3, 19, 20, 22, 24). The mitochondrion, the chloroplast, and the plasmalemma have been implicated as sites of action (1, 6, 21).

Our metabolic data showed that while photosynthetic electron transport is affected by high concentrations of acifluorfen, CO_2-dependent O_2 evolution, which depends on the presence of a functional chloroplast envelope, is inhibited by lower concentrations. These results are consistent with the hypothesis that the DPEs interact with either a stromal or an envelope component of the chloroplast. The envelope acts as a regulator of the interactions between the chloroplast and the cytoplasm, through its low permeability to ionic substances combined with the presence of specific translocators located on the inner envelope membrane (18). A possible site of DPE action is the phosphate translocator, a major constituent of the inner envelope membrane (8). It has been studied both *in vivo* and *in vitro* (9, 11) and operates by a strict counter exchange mechanism, exporting triose-P products of photosynthesis from the stroma and importing Pi from the cytoplasm to maintain the phosphate concentration in the stroma (9).

The finding that photosynthetic electron transport is not as sensitive to the DPEs as are other physiological functions is in accord with the conclusions of others (22, 23). To date no data are available, however, concerning a possible site of action of the DPEs in the chloroplast stroma. The various enzymes whose activities are modulated by light are possible stromal sites. Enzyme activation occurs through the Fd-thioredoxin system which used reducing power supplied by the photosynthetic electron transport system (2, 4). FbPase and GAPDH are activated in this manner. The activation of these enzymes allows carbon fixation to proceed through to the production of triose-P which are then exported out of the chloroplast. Both enzymes are thought to be inactivated through a reversal of the activation pathway with O_2 acting as the terminal oxidant although only FbPase has been directly implicated (26).

The work reported here constitutes an attempt to distinguish between effects of DPEs on the chloroplast envelope and on physiological events which take place in the stroma. Since the action of the herbicide is light-dependent, physiological functions involving a light-mediated step are of the greatest interest in this regard.

MATERIALS AND METHODS

Spinacia oleracea (cv Melody) was grown in a synthetic soil mix in an air-conditioned greenhouse using supplemental lighting from GE metal halide bulbs 400 $\mu E \cdot m^{-2} \cdot s^{-1}$ (16-h photoperiod) during the winter months. Greenhouse temperatures varied from a nighttime low of 21°C to a daytime high of 25°C. Leaves from 3- to 4-week-old plants were used for chloroplast isolation as previously described (1). Thylakoid membranes were prepared from osmotically shocked chloroplasts.

O_2 evolution was followed with a YSI Clark O_2 electrode. Incubations were at 25°C with illumination at 400 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR. Reaction mixtures for CO_2-dependent O_2 evolution contained 0.33 M sorbitol, 50 mM Hepes (pH 7.6), 0.1 mM K_2HPO_4, and 10 mM $NaHCO_3$. Reaction mixtures for ferricyanide-dependent O_2 evolution contained 0.4 M sorbitol, 50 mM Hepes (pH 7.6), 5 mM $MgCl_2$, 10 mM K_2HPO_4, and 1.3 mM $K_3Fe(CN)_6$.

Electron transport was measured using lysed chloroplasts with and without 5 mM NH_4Cl as uncoupler. Chloroplast intactness was measured by the method of Heber and Santarius (14). Reaction mixtures for $^{14}CO_2$ fixation contained 0.33 M sorbitol 50 mM Hepes (pH 7.6), 2 mM EDTA, 0.1 mM Na_2HPO_4, and 10 mM $NaH^{14}CO_3$ (final specific radioactivity, 0.5 mCi/mmol). Reactions were carried out at room temperature under 170 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR for 20 min and were terminated by addition of 20% TCA. The rate of carbon fixation was determined by counting the ^{14}C in acid stable compounds in a 'Liquiscint' (National Diagnostics) cocktail in a Beckman LS-200 liquid scintillation counter. [^{14}C]Acifluorfen uptake was measured by incubating with intact chloroplasts, centrifuging them through silicone oil, and liquid scintillation counting as described above. Chl concentrations were between 40 and 60 μg Chl/ml for all reactions.

Stromal pH was measured by the method of Heldt and Rapley

[1] Supported by a grant from Rohm and Haas Corp. Research Laboratories.

[2] Abbreviations: DPE, diphenylethers; FbPase, fructose 1,6-bisphosphatase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase (NADP).

(15). FbPase was assayed by the method of Rosa (25) and GAPDH by the method of Wu and Racker (28). H_2O_2 was measured by the method of Tanaka *et al.* (27). Chloroplast envelope was purified as previously described (1).

Acifluorfen and [^{14}C]acifluorfen were gifts from Rohm and Haas. NaH$^{14}CO_3$ was from New England Nuclear.

RESULTS

Low concentrations of acifluorfen inhibit CO_2-dependent O_2 evolution in intact spinach chloroplasts, while coupled and uncoupled electron transport are inhibited only at higher concentrations of the herbicide. These findings are summarized in Figure 1, together with data demonstrating the effect of acifluorfen on CO_2-dependent O_2 evolution in a reconstituted chloroplast system. A system involving the intact chloroplast is much more sensitive to acifluorfen action than are thylakoids alone, with a stroma plus thylakoids combination (reconstituted chloroplasts) being intermediate in sensitivity.

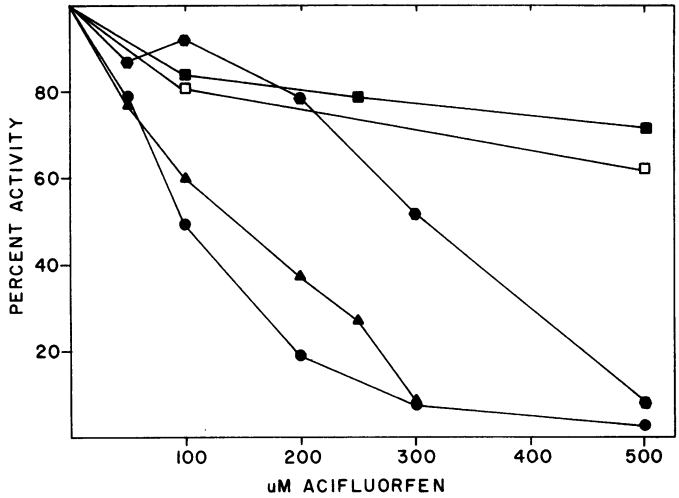

FIG. 1. Effect of acifluorfen on chloroplast functions. (●), CO_2-dependent O_2 evolution; control rate equaled 111.0 μmol O_2/h·mg Chl. (▲), CO_2 fixation; control rate equaled 69 μmol CO_2/h·mg Chl. (●), O_2 evolution, reconstituted system; control rate equaled 73.7 μmol O_2/h·mg Chl. (■), Ferricyanide-dependent O_2 evolution, coupled system; control rate equaled 79.4 μmol O_2/h·mg Chl. (□), Ferricyanide-dependent O_2 evolution, uncoupled system; control rate equaled 408.8 μmol O_2/h·mg Chl. The reconstituted system was supplemented with 0.2 mM Fd, 5 mM PGA, 1 mM Pi, 20 mM MgCl, 0.2 mM ADP, and 0.1 mM NADP.

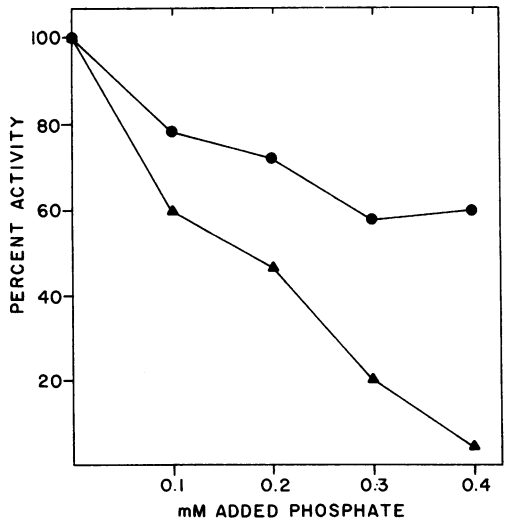

FIG. 2. Effect of acifluorfen on phosphate sensitivity of CO_2-dependent oxygen evolution; control rate equaled 49.3 μmol O_2/h·mg Chl. (●), Control; (▲), 150 μM acifluorfen. CO_2-dependent O_2 evolution was measured as described in "Materials and Methods."

Table I. *Association of Acifluorfen with Isolated Chloroplast Envelope*

[^{14}C]Acifluorfen was added to a purified envelope suspension (1) and centrifuged at 60,000*g* for 2 h (2). The pellet was completely resuspended and centrifuged a second time (3).

Exp.	[^{14}C]Acifluorfen	
	Supernatant	Pellet
	cpm	
1	625	
2	370	386
3	129	111

Table II. *Interaction of Acifluorfen with the Phosphate Translocator*

CO_2-dependent O_2 evolution from intact chloroplasts was measured as described in "Materials and Methods."

Additions	Rate of O_2 Evolution at Following Acifluorfen Levels	
	0 μM	+150 μM
	μmol/mg Chl·h	
Control	37.7	17.4
0.5 mM Pi	14.6	0
0.5 mM Pi, 1.0 mM PPi	36.8	0

Figure 2 shows the effects of added Pi on CO_2-dependent O_2 evolution of intact chloroplasts in the absence and presence of acifluorfen. With acifluorfen present in the reaction mixture, CO_2-dependent O_2 evolution is more sensitive to added Pi. This implicates the phosphate translocator as a site where metabolic perturbation occurs after herbicide treatment. Were phosphate translocation accelerated by acifluorfen, increasing amounts of triose-P would be exported in exchange for increasing inputs of Pi. This in turn would reduce the pool size of intermediates in the stroma and decrease the rate of photosynthesis. This has been demonstrated to take place in isolated chloroplasts by Flügge *et al.* (10).

Other experiments (Table I) indicated that while the herbicide was sequestering itself in the envelope, a simple washing of the envelope preparation was enough to remove the herbicide from it. CO_2-dependent O_2 evolution is partially inhibited by 0.5 mM Pi and completely inhibited by 0.5 mM Pi and 150 μM acifluorfen (Table II). While the inhibition can be overcome in the control system by including 1.0 mM PPi, which acts to inhibit the phosphate translocator (7), the addition of PPi is ineffective at relieving Pi inhibition in the presence of acifluorfen.

These results suggest that acifluorfen does not affect the phosphate translocator directly but rather indirectly. Huber's results (16) suggest an interaction between pH and the functioning of the phosphate translocator. The results in Figure 3 show that the inhibitory effect of acifluorfen on carbon fixation decreases with increasing external pH. To determine whether this result was linked to an effect on stromal pH, we measured the effect of acifluorfen on stromal pH levels attained in the light. The results (Table III) show that acifluorfen did not influence stromal pH.

This data could be explained by a disruption of the physical integrity of the chloroplast envelope by acifluorfen. As shown in Table IV, however, no effect of the DPEs on chloroplast intactness was detected during the time which CO_2-dependent O_2

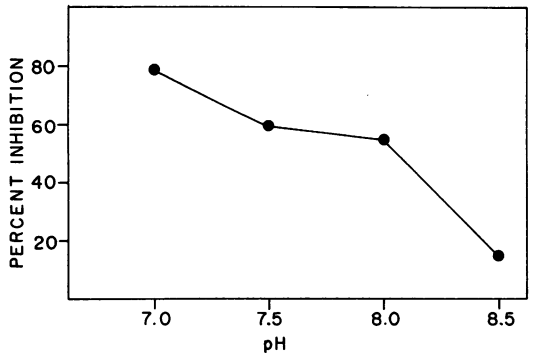

FIG. 3. Effect of pH on acifluorfen inhibition of CO_2-dependent O_2 evolution. CO_2-dependent O_2 evolution was measured as described in "Materials and Methods", with external pH varied as shown. Control rate equaled 102.1 μmol O_2/h·mg Chl. Acifluorfen concentration equaled 150 μM.

Table III. *Measurement of Stromal pH after the Addition of Acifluorfen*

Acifluorfen was added to preparations of intact chloroplasts in concentrations indicated. Stromal pH was measured as described in Heldt and Rapley (15).

Acifluorfen	Stromal pH
μM	
Control	8.17
100	8.29
200	8.33
300	8.28

Table IV. *Effect of Acifluorfen on Intactness of Chloroplasts*

Acifluorfen was added to intact chloroplasts in the concentrations indicated. Percentage intactness was measured as in Heber and Santarius (14).

Acifluorfen	Intactness
μM	%
Control	89
150	90
300	88
500	94

evolution was inhibited (5 min).

Herbicidal activity is light dependent. Our data did not preclude the stroma as a site of light-dependent DPE action. To determine if the herbicide was entering the chloroplast, intact chloroplasts were incubated with [^{14}C]acifluorfen with and without light, centrifuged through silicone oil, and counted to determine the radioactivity contained in the chloroplast pellet. Results (Fig. 4) show that acifluorfen penetration of the chloroplast is light-independent. The effect of acifluorfen on the light-modulated activity of two stromal chloroplast enzymes is shown in Figures 5A and 6A. Addition of acifluorfen to intact chloroplasts after a brief illumination period resulted in subsequent decreases in activity of both FbPase and NADP-GAPDH. Preliminary results demonstrated that FbPase is more rapidly activated than is GAPDH, hence the longer incubation time for the latter enzyme. Because DPE action on intact tissue requires the presence of O_2, the same experiment was also carried out at low oxygen tensions (Figs. 5B and 6B). Addition of acifluorfen under low O_2 tension decreased light-modulated FbPase activity, although this decrease was not immediate as it was in experiments at ambient O_2 levels. NADP-GAPDH was not affected by the presence of acifluorfen under low O_2.

DTT is an artificial substitute for the reductant usually supplied by photosynthetic electron transport for light activation. The ability of acifluorfen to reverse DTT activation of FbPase and NADP-GADPH is shown in Figures 7 and 8. The effects of acifluorfen on light activation are shown for comparison. Activation of FbPase by light and by 30 mM DTT was about the same magnitude, and addition of acifluorfen resulted in the same inhibition of enzyme activity. In the case of NADP-GAPDH, however, addition of acifluorfen resulted only in an initial inhibition of the DTT-induced activation of the enzyme at 30 mM DTT. Addition of 30 mM DTT to acifluorfen-treated chloroplasts in the light partly reversed the inhibitory effect of the herbicide in the case of both enzymes (Fig. 9).

FbPase is inactivated by H_2O_2 in intact chloroplasts (12). H_2O_2 is produced through the dismutation of superoxide radicals by the action of superoxide dismutase which is present in the chloroplast (17). If the presence of acifluorfen resulted in H_2O_2 production, a light-modulated enzyme such as FbPase would be inactivated. Measurements of H_2O_2 levels produced in illumi-

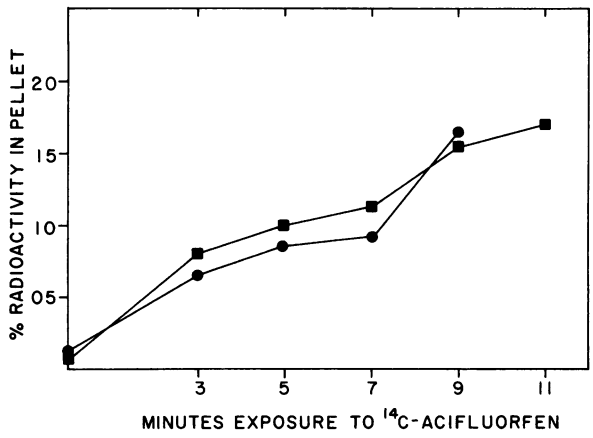

FIG. 4. Entrance of acifluorfen into intact chloroplasts. (●), Light; (■), dark. [^{14}C]Acifluorfen uptake was measured as described in "Materials and Methods."

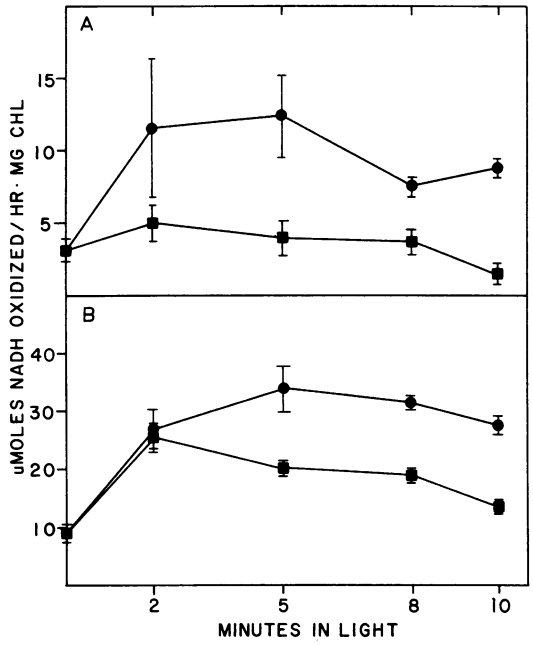

FIG. 5. Effect of acifluorfen on the activation of FbPase by light. (●), Control; (■), 150 μM acifluorfen. A, High oxygen (243 nM); B, low oxygen (18 nM). Acifluorfen was added to intact chloroplasts after 1.75 min incubation in the light.

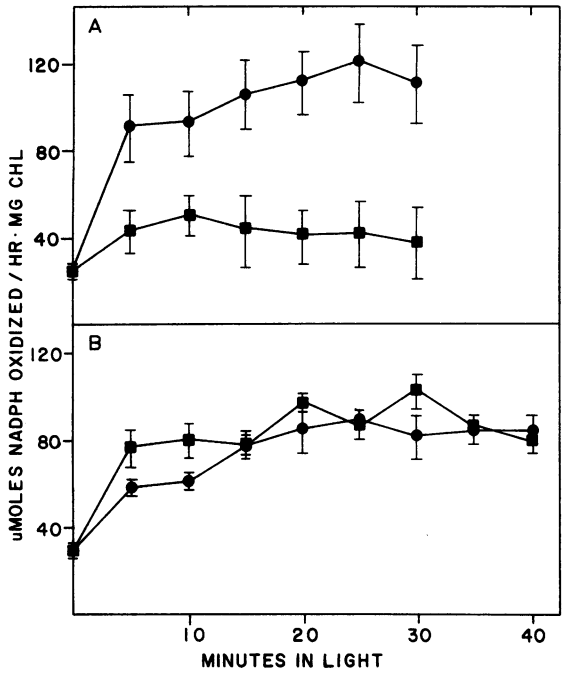

FIG. 6. Effect of acifluorfen on the activation of GAPDH by light. (●), Control; (■), 150 μM acifluorfen. A, High oxygen (243 nM); B, low oxygen (18 nM). Acifluorfen was added to intact chloroplasts after 4.75 min incubation in the light.

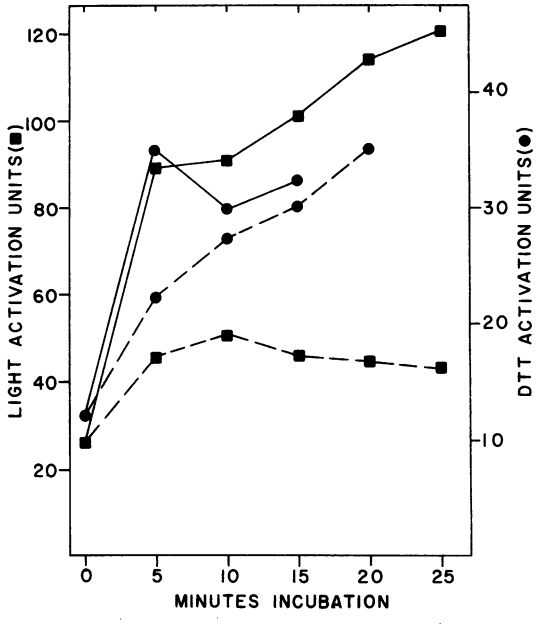

FIG. 8. Effect of acifluorfen on the activation of GAPDH by DTT and light. (■——■), control for light activation; (■- - -■), 150 μM acifluorfen + light; (●——●), control for DTT activation; (●- - -●), 150 μM acifluorfen + DTT. Specific activity units are in μmol NADPH oxidized/h·mg Chl. Acifluorfen was added 5 min after the addition of DTT.

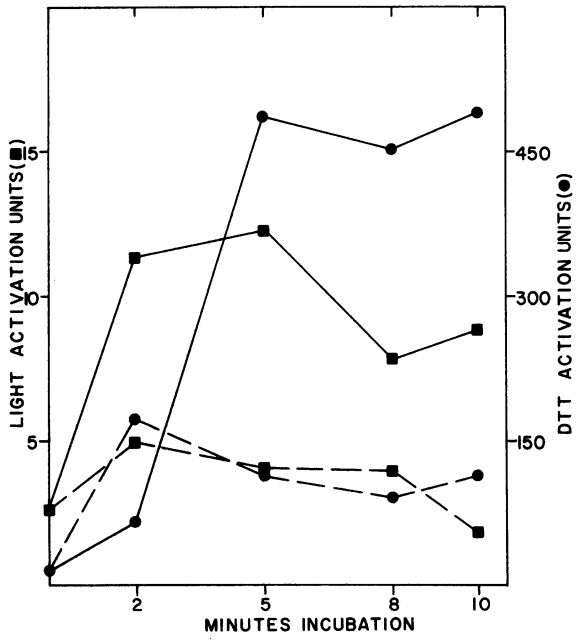

FIG. 7. Effect of acifluorfen on the activation of FbPase by DTT and light. (■——■), Control for light activation; (■- - -■), 150 μM acifluorfen + light; (●——●), control for DTT activation; (●- - -●), 150 μM acifluorfen + DTT. Specific activity units are in μmol NADH oxidized/h·mg Chl. Acifluorfen was added 2 min after the addition of DTT.

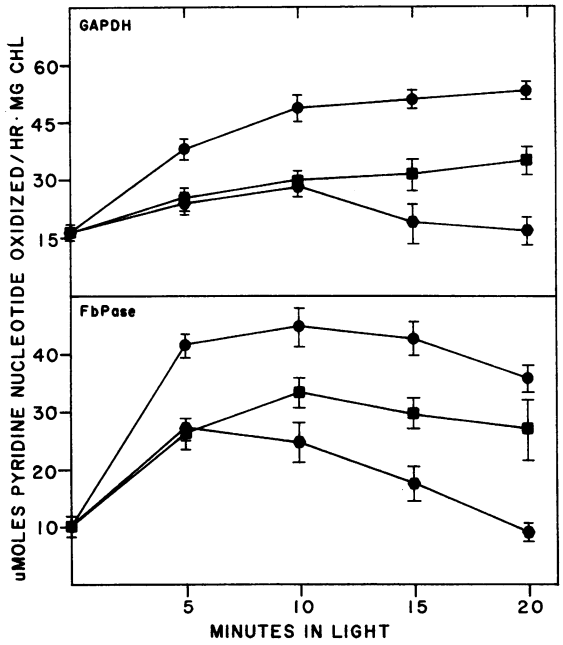

FIG. 9. Activity of acifluorfen-inhibited light-modulated enzymes after addition of DTT. (●), Control; (●), 150 μM acifluorfen; (■), 30 mM DTT + 150 μM acifluorfen. Acifluorfen was added before light incubation of the intact chloroplasts. DTT was added 5 min after light incubation of the intact chloroplasts.

nated chloroplasts in the presence of acifluorfen, however, showed no increase in H_2O_2 production over that of the control. (Control rate equaled 7.28×10^{-5} M H_2O_2 produced/mg Chl·h while in acifluorfen-treated chloroplasts, an increase in H_2O_2 production was not detectable).

DISCUSSION

The data presented here constitute further information concerning the effect of DPE treatment on chloroplast function. Present evidence strongly suggests that electron transport is not a primary site of action of the DPEs in intact spinach chloroplasts. CO_2-dependent O_2 evolution, on the other hand, is affected by the herbicide. The results presented here enable us to distinguish possible effects on the envelope from those exercised on stromal function. A DPE-induced sensitivity of CO_2-dependent O_2 evolution to added phosphate could be due to an effect

on the envelope's phosphate translocator. Alternatively, it could be the consequence of a slowing of carbon fixation which would, in turn, result in an increased sensitivity of that process to added phosphate. The modulation of the stromal enzymes, FbPase, and GAPDH activities by light were chosen for study as possible targets of acifluorfen action since our evidence to date had not ruled out light-dependent physiological functions in the stroma as sites of DPE activity. The activities of two light-modulated enzymes, FbPase and NADP-GAPDH, were much decreased by the herbicide (Figs. 5A and 6A). Acifluorfen had the same inhibitory effect on FbPase activity whether the source of reductant was exogenously added DTT or photosynthetic electron transport in illuminated chloroplasts (Fig. 7).

Acifluorfen only transiently inhibited GAPDH activation by DTT (Fig. 8). The effect of acifluorfen on the activities of FbPase and GAPDH also differed at low O_2 tensions. The herbicide was ineffective in inhibiting GAPDH activation at low O_2 tensions, whereas the activation of FbPase was affected by acifluorfen under low O_2 tensions (Figs. 5B and 6B) although not to the same extent as under ambient conditions. It is possible that sufficient O_2 was produced during the initial stages of the illumination period to affect FbPase activity but not enough to influence the activity of GAPDH. Schürmann (26) found that even under anaerobic conditions the inactivation of FbPase was slowed by the inclusion of an O_2 trap in the reaction mixture, suggesting that very low levels of O_2 are sufficient to elicit FbPase inactivation. Both control and acifluorfen-treated chloroplasts exhibited higher rates of enzyme activity at low O_2 tensions (Figs. 5 and 6).

The activation of FbPase and GAPDH by both light and DTT is either permanently or transiently reversed by acifluorfen. The pathway responsible for modulation of the activities of these two enzymes is the Fd-FTR-thioredoxin pathway, whether the source of reductant is photosynthetic electron transport in the case of light activation or DTT. It seems plausible, therefore, to propose that the Fd-FTR-thioredoxin pathway is a site of action of acifluorfen within the chloroplast.

The peroxidation of thylakoid lipids has been demonstrated to take place as a result of acifluorfen action under certain conditions (19). The perturbation of the thylakoid structure is associated with inhibitory effects on photosynthetic electron transport (3). We observe no effect of acifluorfen on photosynthetic electron transport at concentrations which inhibit CO_2-dependent O_2 evolution and which reverse activation of FbPase and GAPDH. It is, therefore, unlikely that these effects have their primary cause in a perturbation of thylakoid lipids through peroxidation. Envelope intactness was also unaffected by acifluorfen (Table IV). If the presence of the herbicide at the concentrations used by us were resulting in a general destructive process through peroxidation, the effects of that process would most likely be expressed at either the thylakoids or the envelope. This was not the case with the results reported here.

We propose that acifluorfen treatment directly or indirectly stimulates the pathway for enzyme inactivation and thus causes the inhibition of carbon fixation. The scheme of Schürmann (26) for the regulation of chloroplast FbPase is a useful model in this context. He proposed that the terminal oxidant for FbPase inactivation is O_2. DPE action is known to require the presence of O_2 (3). Our results, showing the effect of lowered O_2 tension on the inactivation of the enzymes by acifluorfen, are also in accord with this requirement. His data also show that at higher pH (7.9) FbPase forms a stable complex with its substrate, rendering it less susceptible to the inactivation pathway whose terminal oxidant is O_2. This is compatible with our results demonstrating the decreased effectiveness of acifluorfen in inhibiting CO_2-dependent O_2 evolution at higher pH (Fig. 3). H_2O_2 is not involved in this inactivation pathway. Our data also demonstrated no effect of acifluorfen on H_2O_2 production in illuminated chloroplasts.

The addition of DTT after acifluorfen to illuminated chloroplasts lessened the inhibition by the herbicide of both enzymes. DTT provides reductant to the activation pathway at the level of thioredoxin. If acifluorfen acted on thioredoxin or at a site closer to the terminal oxidant than that molecule, this could result in the results reported here.

The data presented here, as well as previous work from our laboratory (1) and from elsewhere (23), are all compatible with the hypothesis that acifluorfen stimulates a pathway for the inactivation of light-activated chloroplast enzymes, resulting in an inhibition of carbon fixation. Acifluorfen, at the concentrations used here, does not affect photosynthetic electron tranport. In the presence of acifluorfen, therefore, electron transport would continue, and carbon fixation would become inhibited as FbPase and GAPDH were inactivated. Reductant produced as a result of electron transport would be shunted to the reduction of molecular oxygen in the Mehler reaction (13). Superoxide radical levels would increase with attendant increases in more reactive species, such as OH·, which are known to cause peroxidations of membrane lipids (13). Lipid peroxidation is a well-documented consequence of DPE action (3, 22).

We propose, therefore, that a toxic action of DPE treatment within the chloroplast is an effect on the Fd/Fd-thioredoxin reductase/thioredoxin pathway which acts to regulate the activity of key enzymes of carbon fixation. Further work is needed to determine the relationship of this chloroplast site to those located outside it, at the plasmalemma or in the mitochondrion.

LITERATURE CITED

1. ALSCHER R, C STRICK 1984 Diphenyl ether-chloroplast interactions. Pestic Biochem Biophys 21: 248–255
2. ANDERSON LE 1979 Interaction between photochemistry and acitivity of enzymes. *In* M Gibbs, E Latzko, eds, Encyclopedia of Plant Physiology: New Series, Vol 6. Springer-Verlag, Berlin, pp 271–281
3. BÖGER P 1984 Multiple modes of action of diphenyl ethers. Z Naturforsch 39C: 468–475
4. BUCHANAN BB 1980 Role of light in the regulation of chloroplast enzymes. Annu Rev Plant Physiol 31: 341–347
5. DEVLIN RM, SJ KARCZMARCZYK, II ZBEIC 1983 Influence of Norflurazon on the activation of substituted diphenylether herbicides by light. Weed Sci 31: 109–112
6. DUKE SO, KC VAUGHN, RL MEEUSEN 1984 Mitochondrial involvement in the mode of action of acifluorfen. Pestic Biochem Physiol 21: 368–376
7. FLIEGE R, UI FLÜGGE, K WERDAN, HW HELDT 1978 Specific transport of inorganic phosphate, 23-phosphoglycerate and triosephosphate across the inner membrane of the envelope in spinach chloroplasts. Biochim Biophys Acta 502: 232–247
8. FLÜGGE UI, HW HELDT 1977 Specific labeling of a protein involved in phosphate transport of chloroplasts by pyridoxal-5'-phosphate. FEBS Lett 82: 29–33
9. FLÜGGE UI, HW HELDT 1978 Specific labeling of the active site of the phosphate translocator in spinach chloroplasts by 2,4,6-trinitrobenzene sulfonate. Biochem Biophys Res Commun 84: 37–44
10. FLÜGGE UI, M FREISL, HW HELDT 1980 Balance between metabolic accumulation and transport in relation to photosynthesis in isolated spinach chloroplasts. Plant Physiol 65: 574–577
11. FLÜGGE UI, HW HELDT 1981 The phosphate translocator of the chloroplast envelope: Isolation of the carrier protein and reconstitution of transport. Biochim Biophys Acta 638: 296–304
12. FOYER C, J ROWELL, DA WALKER 1983 Measurement of the ascorbate content of spinach leaf protoplasts and chloroplasts during illumination. Planta 157: 239–244
13. HALLIWELL B 1981 Chloroplast Metabolism. Clarendon Press, Oxford
14. HEBER U, KA SANTARIUS 1970 Direct and indirect transfer of ATP and ADP across the chloroplast envelope. Z Naturforsch 25B: 718–728
15. HELDT HW, L RAPLEY 1970 Specific transport of inorganic phosphate, 3-phosphoglycerate and dihydroxyacetone phosphate and of dicarboxylates across the inner membrane of spinach chloroplasts. FEBS Lett 10: 143–148
16. HUBER SC 1979 Effect of pH on chloroplast photosynthesis: Inhibition of O_2 evolution by inorganic phosphate and magnesium. Biochim Biophys Acta 545: 131–140
17. JACKSON C, J DENCH, AL MOORE, B HALLIWELL, CH FOYER, DO HALL 1978 Subcellular localisation and identification of superoxide dismutase in the leaves of higher plants. Eur J Biochem 91: 339–344

18. KRAUSE GH, U HEBER 1976 Energetics of intact chloroplasts. *In* J Barber, ed, Topics of Photosynthesis, Vol 1. Elsevier/North Holland Biomedical Press, Amsterdam, pp 171–214
19. KUNERT KJ 1984 The diphenyl-ether herbicide oxyfluorfen: a potent inducer of lipid peroxidation in higher plants. Z Naturforsch 39C: 476–481
20. LAMBERT R, G SANDMANN, P BÖGER 1983 Mode of action of nitrodiphenylethers affecting pigments and membrane integrity. *In* J Miyamoto, PC Kearney, eds, Pesticide Chemistry, Human Welfare and the Environment, Vol 3. Pergamon Press, Oxford, pp 97–102
21. LEONG TY, WR BRIGGS 1982 Evidence from studies with acifluorfen for participation of a flavin-cytochrome complex in blue light. Plant Physiol 70: 874–881
22. ORR GL, FD HESS 1981 Characterization of herbicidal injury by acifluorfen-methyl in excised cucumber (*Cucumis sativus* L.) cotyledons. Pestic Biochem Physiol 16: 171–178
23. ORR GL, FD HESS 1982 Mechanism of action of the diphenyl ether herbicide acifluorfen-methyl in excised cucumber (*Cucumis sativus* L.) cotyledons. Plant Physiol 69: 502–507
24. ORR GL, FD HESS 1982 Proposed site(s) of action of new diphenyl ether herbicides. ACS Symp Ser 181: 131–152
25. ROSA L 1981 The rapid activation *in vitro* of the chloroplast fructose-1,6-bisphosphatase followed using a new assay procedure. FEBS Lett 134: 151–154
26. SCHÜRMANN P, Y KOBAYASHI 1984 Regulation of chloroplast fructose 1,6-bisphosphatase activity by the ferredoxin/thioredoxin system. *In* C Sybesma, ed, Advances in Photosynthesis Research, Proceedings of the 6th International Congress on Photosynthesis (1983) Vol 3. Nijhoff/Junk Publishers, The Hague, pp 629–632
27. TANAKA K, N KONDO, K SUGAHARA 1982 Accumulation of hydrogen peroxide in chloroplasts of SO_2-fumigated spinach leaves. Plant Cell Physiol 23: 999–1007
28. WU R, E RACKER 1959 Regulatory mechanisms in carbohydrate metabolism. III. Limiting factors in glycolysis of *Ascites* tumor cells. J Biol Chem 234: 1029–1035