Short Communication

Inhibition of Photophosphorylation by 1,1,1-Trichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDT)

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The reaction of barley to DDT† is controlled by a single major gene with susceptibility dominant to resistance (Hayes, 1959; Wiebe & Hayes, 1960). The susceptible barley varieties rapidly develop a severe chlorosis when they come into contact with DDT whereas resistant varieties are not affected. [Analogues of DDT with (a) methoxyl, chlorine, bromine or fluorine in the *para* positions of the phenyl rings, (b) the C-2 ethane hydrogen and (c) the trichloro- or tribromoethane grouping are also toxic to some varieties of barley (Upshall & Goodwin, (1964).]

We have previously shown (Lawler & Rogers, 1967) that DDT affects the photosynthesis of susceptible barley. Within 6 hr. after spraying susceptible barley with DDT there was a decrease in the rate of O₂ evolution; O₂ evolution progressively decreased as chlorotic symptoms became evident. Chloroplasts isolated from DDT-treated susceptible barley showed diminished Hill activity, as measured by the photoreduction of 2,6-dichlorophenol-indophenol, compared with chloroplasts from DDT-treated resistant barley or from untreated plants of either type. It has also been shown (Wurster, 1968) that DDT will inhibit photosynthesis (CO₂ assimilation) in phytoplankton. The further experiments reported here demonstrate that DDT inhibits cyclic photophosphorylation in susceptible barley but appears to have no effect on non-cyclic photophosphorylation.

Experimental methods. Chloroplasts were prepared from primary leaves of 6–8-day-old barley plants by a procedure adapted from that of Kleese (1966). Ascorbate was omitted from the isolation medium when non-cyclic photophosphorylation was to be measured. Chlorophyll content was determined by the method of Arnon (1949).

Cyclic photophosphorylation was measured by

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† Abbreviations: DDT, 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane; DDE, 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethylene.

determining the decrease in P_i that occurred when the chloroplasts were incubated in the reaction mixture for 3 min. at 26° in the light (14 000 ft.-candles). The reaction mixture contained 45 μmoles of tris-HCl buffer, pH 8.2, 60 μmoles of NaCl, 12 μmoles of MgCl₂, 5 μmoles of KH₂PO₄, 10.3 μmoles of ADP and 0.1 μmole of phenazine methosulphate in a final volume of 2.5 ml. in a 1 cm.-light-path glass cuvette. The chlorophyll concentration was approx. 80 μg./cuvette. The reaction mixture was inactivated by adding trichloroacetic acid. P_i was determined by the method of Fiske & Subbarow (1925). In one experiment the phenazine methosulphate was replaced by 0.25 μmole of diquat. Non-cyclic photophosphorylation was measured by following the photoreduction of ferricyanide and by determining the decrease in P_i that accompanied photoreduction of ferricyanide when the chloroplasts were incubated in the reaction mixture for 10 min. at 26° in the light (14 000 ft.-candles). The reaction mixture contained 45 μmoles of tris-HCl buffer, pH 8.35, 60 μmoles of NaCl, 12 μmoles of MgCl₂, 5 μmoles of KH₂PO₄, 10.3 μmoles of ADP and 1.5 μmoles of K₃Fe(CN)₆ in a final volume of 3.0 ml. in a 1 cm.-light-path glass cuvette. The chlorophyll

DDT

DDE

concentration was approx. 50 $\mu\text{g.}/\text{cuvette}$. Photo-reduction of ferricyanide was followed by decrease in the extinction at 420 $\text{m}\mu$. The reaction mixture was inactivated by adding trichloroacetic acid, and P_1 was determined by the method of Berenblum & Chain (1938).

Pseudo-cyclic photophosphorylation was identified by determining the decrease in P_1 that occurred when the chloroplasts were incubated in a reaction mixture identical with that used to study cyclic photophosphorylation but with the addition of 3-(*p*-chlorophenyl)-1,1-dimethylurea (final concn. 0.1 mm).

Two varieties of barley were used in the investigations: Rika, a variety susceptible to DDT, and Proctor, a resistant variety. The plants were sprayed at the two-leaf stage with a DDT emulsion (0.5 g. of DDT in 20 ml. of acetone, then water to 200 ml.) delivered from a spray operated by an aerosol power-pack. In all experiments control plants were sprayed with an acetone-water mixture not containing DDT. In other studies chloroplasts isolated from untreated plants were treated with 200 $\mu\text{g.}$ of DDT (in 10% acetone)/mg. of chlorophyll (approx. 1.0 ml. of chloroplast suspension).

Results. (a) Effect of DDT on cyclic photophosphorylation. Chloroplasts isolated from DDT-treated susceptible barley were found to have very low rates of phenazine methosulphate catalysed cyclic photophosphorylation compared with those from treated resistant barley and from untreated plants (Table 1). Corresponding results were obtained if chloroplasts were isolated from untreated barley and then allowed to come into contact with DDT (200 $\mu\text{g.}/\text{mg.}$ of chlorophyll) for 60 min. before measurement of photophosphorylation rates.

Similarly diquat-catalysed cyclic photophosphorylation in chloroplasts from susceptible barley was decreased by treatment with DDT from 170 to less than 10 μmoles of $P_1/\text{hr.}/\text{mg.}$ of chlorophyll. In

the presence of 0.1 mm -3-(*p*-chlorophenyl)-1,1-dimethylurea the phenazine methosulphate-catalysed cyclic photophosphorylation of chloroplasts isolated from susceptible barley was decreased by treatment with DDT from 200 to 60 μmoles of $P_1/\text{hr.}/\text{mg.}$ of chlorophyll. Preliminary studies had shown that this concentration of 3-(*p*-chlorophenyl)-1,1-dimethylurea was sufficient to inhibit almost entirely the reduction of 2,6-dichlorophenol-indophenol by chloroplasts from susceptible barley.

(b) Effect of DDT on non-cyclic photophosphorylation. Preliminary investigation showed that, in contrast with the photoreduction of 2,6-dichlorophenol-indophenol, the photoreduction of ferricyanide by chloroplasts from DDT-treated barley was the same in both susceptible and resistant varieties and approximated to the rates achieved by chloroplasts from untreated barley (40 μmoles of ferricyanide reduced/hr./mg. of chlorophyll. Neither photoreduction of ferricyanide nor non-cyclic photophosphorylation by chloroplasts from barley was affected by treatment with DDT (Table 1).

(c) Effect of an analogue of DDT on cyclic photophosphorylation. The effect of DDE, an analogue of DDT that is not toxic to either variety of barley (Upshall & Goodwin, 1964; Wiebe, 1963), on cyclic photophosphorylation by susceptible barley was examined. Exposure of chloroplasts from susceptible barley to DDE (200 $\mu\text{g.}/\text{mg.}$ of chlorophyll) for 60 min. had no effect on cyclic photophosphorylation, whereas under the same conditions DDT inhibited cyclic photophosphorylation by 100%. Moreover, the effect of DDT on cyclic photophosphorylation by chloroplasts from susceptible barley could be significantly decreased by suspension of the chloroplasts in a medium containing DDE before they were exposed to DDT. Thus exposure of chloroplasts from susceptible barley to DDE for 60 min. and then to DDT for 60 min. led to only 13% inhibition of cyclic photo-

Table 1. *Effect of DDT on cyclic and non-cyclic photophosphorylation in barley*

Expts. (a), (c) and (d) were carried out on chloroplasts treated with DDT *in vitro*; the chloroplasts used in Expt. (b) were isolated from DDT-treated seedlings. Experimental details are given in the text.

Source of chloroplasts ...	Susceptible barley		Resistant barley	
	DDT absent	DDT present	DDT absent	DDT present
(a) Cyclic photophosphorylation (μmoles of $P_1/\text{hr.}/\text{mg.}$ of chlorophyll)	390	45	530	530
(b) Cyclic photophosphorylation (μmoles of $P_1/\text{hr.}/\text{mg.}$ of chlorophyll)	340	< 10	480	315
(c) Ferricyanide reduction ($\mu\text{moles}/\text{hr.}/\text{mg.}$ of chlorophyll)	54	52	70	57
	55	54	78	69
(d) Non-cyclic photophosphorylation (μmoles of $P_1/\text{hr.}/\text{mg.}$ of chlorophyll)	58	93	47	58
	36	47	59	59

phosphorylation, whereas exposure to DDT for 60 min. and then to DDE for 60 min. led to 100% inhibition.

(d) Discussion. The results reported here indicate that in susceptible barley DDT inhibits cyclic photophosphorylation but has no effect on photo-reduction of ferricyanide or associated non-cyclic photophosphorylation. Since DDT is toxic to susceptible barley the results emphasize the importance of cyclic photophosphorylation in photosynthesis *in vivo*. That a precise stereochemical conformation is responsible for the observed inhibition of cyclic photophosphorylation by DDT is indicated by the observation that DDE, a structural analogue of DDT, has no effect on photophosphorylation in susceptible barley. The finding that DDE can protect chloroplasts of susceptible barley against the effect of DDT suggests that both compounds possess the complementary conformations necessary for attachment to the 'target' site in a lamellar membrane. However, by reason of its

functional groups only DDT can affect the process of cyclic photophosphorylation.

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