Biochemistry of Herbicides Affecting Photosynthesis

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Herbicides, Photosynthesis, Pigment Synthesis, Inhibition

This paper reviews the inhibitory effects of herbicides at three locations within photosynthetic electron transport, on photophosphorylation, and on pigment synthesis.

1. Introduction

This paper is an introduction to the primary effects of herbicides on photosynthetic electron transport, photophosphorylation and pigment synthesis. Herbicides are not known to have primary effects on transcription, translation or carbon fixation, and the inhibition of lipid synthesis by the thiolcarbamates will not be considered since it is not restricted to the chloroplast. We will be concerned mainly with the primary biochemical effects of compounds so far as they are known, since reports of gross effects on whole plants do not provide leads which can be of use in seeking out new active herbicides. We will need to concentrate on the most recent developments; a previous review [1] considers the earlier work. Throughout we shall be emphasizing information that might aid the synthesis of new herbicides.

2. Compounds interfering with electron transport (Fig. 2)

Most herbicides in use today kill their target weeds by interaction with photosynthetic electron transport [2, 3], summarized in Fig. 1 [4]. For a summary of the minority views on the organisation of electron transport see [5]. The heavy arrows indicate the three sites we shall consider.

2.1. The DCMU site

Many classes of compound are now know to inhibit the Hill reaction (electron transfer between H_2O and an electron acceptor acting before or after photosystem I, usually DCPIP or ferricyanide in an uncoupled system). Examples of the well known classes are given by Draber *et al.* [2]. The common structural requirements for activity in many chemical series have been pointed out [2, 6–8]; essentially inhibitors have a lipophilic moiety near the group $-C(=X)-N=$ ($X=O$ or NH). One of the substituents on the nitrogen is often H but this is not obligatory [2]. Some exceptions to these simple criteria will be mentioned later.

Location. The DCMU site is close to photosystem II (see [1] for summary of early evidence) and most workers believe that DCMU blocks electron transfer from the primary acceptor of photosystem II (Q) to plastoquinone [9] though there may also be an effect on the water-splitting reaction [10]. Much progress on the detailed location is reported elsewhere in this volume. The hatched area in Fig. 1 indicates the proteinaceous shield which is part of a model of the acceptor side of photosystem II developed by Renger [11]. He suggests that DCMU interacts at this site by binding to the protein, because once the shield had been digested with trypsin, several inhibitory effects of DCMU were no longer seen. Other research groups have now examined additional electron transport inhibitors using this technique. All "DCMU-site" inhibitors behave in the same way; trifluralin (**1**), nitrofen (**3**), and perhaps ioxynil (**5**) do not (see [12] and other papers in this volume).

Direct evidence that many Hill reaction inhibitors bind at the same site has recently been obtained in the elegant experiments of Tischer and Strottman [13]. They studied the binding of radioactive atrazine (**7**), metribuzin (**9**) and phenmedipham (**10**) to chloroplast fragments and established (i) that these compounds and DCMU compete directly with one another for the same site and (ii) that the binding constants calculated directly from studies with radioactive compound or indirectly from competi-

Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; DCPIP, dichlorophenolindophenol; DNOC, 4,6-dinitro-*o*-cresol; EPTC, ethyl-N,N-di-*n*-propylthiolcarbamate.

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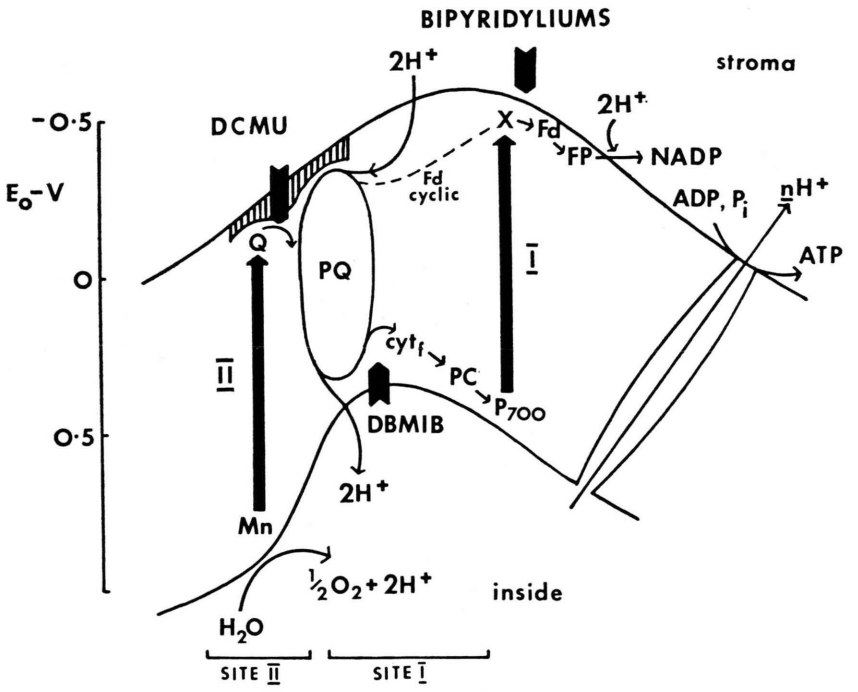


Fig. 1. Diagrammatic representation of the electron transport pathway of photosynthesis. See, for instance, reference [4] for further details and sources. The sites considered in the text are marked.

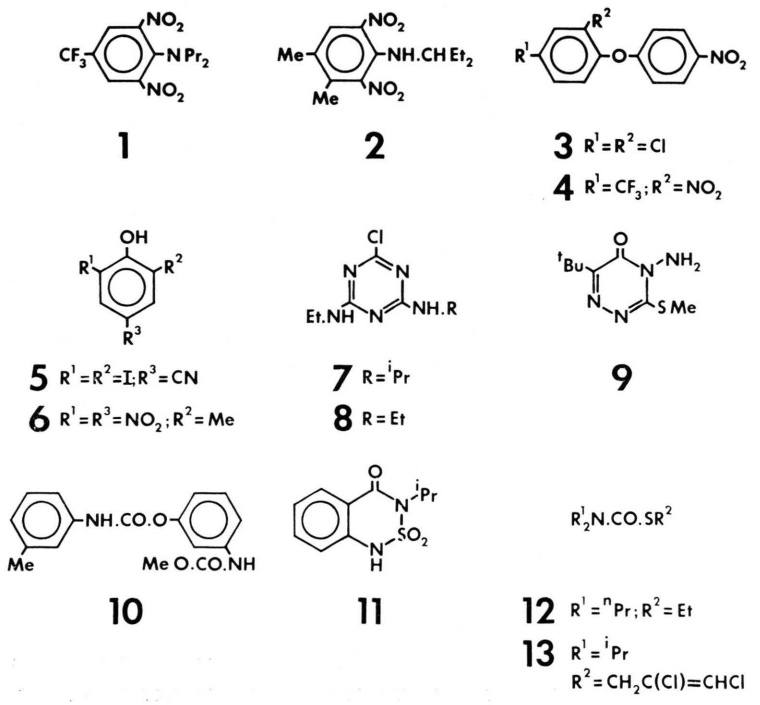


Fig. 2. Structures of compounds interfering with electron transport (see text). **1**, trifluralin; **2**, penoxalin; **3**, nitrofen; **4**, fluorodifen; **5**, ioxynil; **6**, DNOC; **7**, atrazine; **8**, simazine; **9**, metribuzin; **10**, phenmedipham; **11**, bentazon; **12**, EPTC; **13**, diallate.

tion experiments were the same as the inhibition constants computed from Hill reaction inhibition data. Hence this site at which all the compounds compete is almost certainly the site at which inhibition is brought about.

Information about the DCMU site has recently been provided by Machado and Arntzen *et al.* ([14], and see this volume). They discovered that triazine resistance displayed by the weed *Chenopodium album* was due to a change in the chloroplast binding site so that binding of atrazine (**7**) was greatly decreased whilst DCMU binding was unaffected. Taken together with the results described above it seems likely that the various classes of compound bind to different overlapping parts of a protein receptor site so as to bring about inhibition of electron transfer [15].

Compounds which might inhibit the Hill reaction. Although bentazon (**11**) has been shown to be a Hill reaction inhibitor [16], it was less effective than the inhibitors considered so far (*e. g.* 50% inhibition of methyl viologen reduction at 200 μM) and the inhibition was, unusually, progressive with time; Böger *et al.* [17] suggested from their work on algal chloroplasts and whole algae that a metabolite may have been responsible.

Van Rensen *et al.* [18] measured the effects of DNOC (**6**) on the Hill reaction using a variety of conditions. The uncoupled ferricyanide Hill reaction was the most sensitive, 50% inhibition being caused at about 1 μM with uncoupling effects at higher concentrations. Trebst and Draber [15] have recently carried out regression analyses on Hill reaction inhibition results from a considerable number of 2,6-disubstituted-4-nitrophenols and have shown that the shape of the substituents was important rather than their hydrophobic parameters. The authors postulate that the nitrophenols bind to a different site on the shielding protein (from DCMU and other Hill reaction inhibitors) although the same inhibitory effect on electron transport is induced.

Diphenyl ethers also inhibit the Hill reaction, but the reported I_{50} values are perhaps a little high (nitrofen (**3**) 22 – 23 μM; but *cf.* fluorodifen (**4**), 4.5 – 5.6 μM; [19]), and, like the dinitrophenols, they do not fit the structural requirements for the DCMU site. Further, van Assche [12] found that nitrofen did not compete with labelled DCMU for binding to chloroplast membranes. Nitrofen and, to a lesser extent, fluorodifen are modest inhibitors of plant mitochondrial electron transport (I_{50} for nitrofen 27 – 74 μM, depending on the substrate; [19]), so this might contribute to their herbicidal effect. A further suggestion is that the compound acts as an energy-transfer inhibitor (see section 3).

Reason why plants are killed. Although inhibition at the diuron site would cause depletion of ATP and $NADPH_2$ death is probably not due to "starvation" but rather to damage following a failure of the mechanism that normally protects the photosynthetic apparatus against excessive illumination [20]. Stanger and Appleby [21] suggested that the damage was initiated by the lack of $NADPH_2$ required to maintain the protective carotenoid pigments in the reduced form. Ridley [20], however, suggests that when DCMU blocks electron transport conformational changes in the membrane that allow spillover of excitation energy from the antennae units serving PS II to those serving PS I are prevented. Protection via the normal mechanism involving carotenoids is then no longer adequate and pigment breakdown ensues. When electron transport between the photosystems resumes following the addition of, *e. g.* a donor between the photosystems, the conformational change which allows spillover can occur again and protection is restored.

Both schemes are consistent with the results of Moreland and Hill [3] who incubated isolated chloroplasts with DCMU in the light and in the dark, and then washed the chloroplasts to determine if any irreversible inhibition had occurred. Less activity was restored to chloroplasts kept in the light, presumably due to pigment breakdown. However, when the experiment was conducted with simazine (**8**) the same restoration of activity was found after both treatments. If both these compounds do bind to the same site [13], the precise cause of death may not be the same in each case; this warrants further investigation.

2.2. *The action of the bipyridylium herbicides*

The basic mechanism of action of these compounds has been quite well understood for some time although the details of the toxic species and their effects are still under investigation. The herbicides interact with photosynthetic electron transport as indicated in Fig. 1. Structural requirements for activity are known [22] and the sequence of events occurring after the primary interception of an elec-

tron has been summarised [1, 23]. The reduced molecule (paraquat$^{+\cdot}$) is autoxidisable using molecular oxygen to form the superoxide ion ($O_2^{-\cdot}$) which survives long enough to be the toxic species. It can react further to generate hydrogen peroxide which is also a candidate for the toxic product within the plant. In the course of the reaction, the bipyridylium cation is regenerated so that the cycle can begin again. No commercially successful herbicide of this class has been introduced since 1964.

2.3. *The action at the DBMIB site*

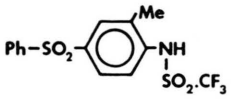
The DBMIB site (Fig. 1) has been left until last since no commercial herbicide has yet been shown to act principally at the photosystem I side of plastoquinone [9]. Recently, in their paper on the dinitrophenol Hill reaction inhibitors Trebst and Draber [15] reported that certain related diphenyl ethers with herbicidal activity do in fact inhibit at this step (though this was not true of them all). Thus it still seems possible that a herbicide acting at this site may be discovered.

We mentioned earlier that the thiolcarbamate herbicides are thought to exert their main action through an inhibition of lipid synthesis [1]. There have however been two recent reports of the effects of EPTC (**12**) and diallate (**13**) on chloroplast functions. EPTC was shown to influence the ratio of reduced to oxidised plastoquinone in sand-grown wheat plants [24] and diallate affected electron transport at high concentrations (around 30 μM) with complex effects that in some ways were like those of DBMIB [25].

New members of the dinitroaniline class of herbicides (*e. g.* penoxalin, (**2**)) have been introduced during the past 5 years. Trifluralin (**1**), the most studied with respect to its mode of action, inhibits the Hill reaction at concentrations in the range 11 – 30 μM [25, 26]. The effects are similar to, though not identical with, those of DBMIB. These concentrations are high for the primary mode of action of a commercial compound, and have to be compared with effects on cell division at concentrations as low as 1 nM [27]. Cell division is therefore still the best candidate for the primary site of action of the dinitroanilines [1].

3. Photophosphorylation (Fig. 3)

During light-driven electron flow, protons are moved inside the thylakoids and create a pH gradient which drives the synthesis of ATP (according to the chemiosmotic theory; see [28]). Compounds can interfere with coupled photophosphorylation either by uncoupling it (by dissipating the pH gradient or by interfering directly with the coupling process)



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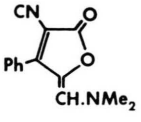

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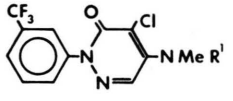
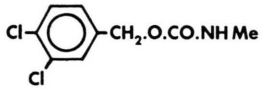


16 R^1=Me

17 R^1=H



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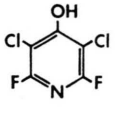

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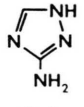

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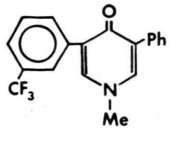

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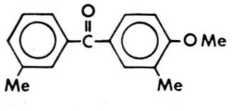

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Fig. 3. Structures of compounds interfering with photophosphorylation and pigment synthesis. **14**, perfluidone; **15**, difunone; **16**, San 6706; **17**, norflurazon; **18**, dichlormate; **19**, haloxydine; **20**, aminotriazole; **21**, fluridone; **22**, methoxyphenone.

or through inhibition of the synthesis of ATP ("energy-transfer inhibition").

There is no pesticide known to act solely through its effects on photophosphorylation, though this seems to be the only reported effect of perfluidone (**14**). This compound gave 20 – 70% inhibition of non-cyclic photophosphorylation at 10 – 50 μm in spinach chloroplasts, while not inhibiting electron transport [29].

Recent work by Lambert *et al.* [30] has raised the possibility that nitrofen (**3**) might be an energy-transfer inhibitor in *Bumilleriopsis* chloroplasts, but this has not been established as its primary herbicidal effect in higher plants.

Some recent compounds affecting photophosphorylation [31, 32] have not been commercialised. Thus uncoupling or inhibition of photophosphorylation is still only a promising site for herbicide action, rather than an established one. At Fisons we attempted to make herbicides by designing and optimising photophosphorylation uncouplers [33]. In fact the action of the most effective herbicides was best explained by their action on carotenoid biosynthesis (section 4).

4. Pigment synthesis (Fig. 3)

Chlorophyll. The experimental herbicide difunone (**15**) inhibits plastid development so that neither chlorophyll nor thylakoid membrane can accumulate and this led to a search for an effect on chlorophyll synthesis. Hampp *et al.* [34] examined a number of enzymes in the chlorophyll biosynthetic pathway and found that urogen I synthetase/urogen III synthetase was inhibited 40% after growth of seedlings of the tropical grass *Pennisetum typhoides* on 40 μm difunone. However Urbach *et al.* [35] have since found that 1 μM difunone caused severe inhibition of carotenoid biosynthesis in the alga *Ankistrodesmus braunii* (see next section).

Carotenoids. There is no shortage of work examining the effects of, principally, the pyridazinones on the pigment content of treated plants. Most has been done with San 6706 (**16**) which is converted to norflurazon (**17**) *in vivo* ([36] and *loc. cit.*, [37]). Although plants probably vary in their ability to metabolise San 6706, and some different effects of the two compounds have been noted [38] we will make the simplifying assumption that studies with these compounds can be taken as equivalent.

The fact that carotenoid biosynthesis is inhibited cannot be disputed; β-carotene formation is lowered and various precursors accumulate, usually phytoene [39, 40] and also phytofluene [41, 42]. Following the loss of carotenoids the accompanying decrease in chlorophyll [36] is easily explained. Other processes are also affected. St. John and Hilton [38] noted effects on the degree of saturation of the fatty acids in polar lipids (*e. g.* galactolipids) of wheat shoots caused by norflurazon and San 6706. Also Blume and McClure [43] found increased levels of phenylalanine ammonia lyase and polyphenolics in barley seedlings treated with San 6706, but the effects were complex and not open to any simple conclusions. The demonstration that chloroplast ribosome formation in the light is inhibited by San 6706 [44] can be ruled out as a possible unifying theory of the mode of action of the pyridazinones since it does not happen in the dark; it is therefore more likely to be a secondary effect [45, 46].

We can speculate about the sequence of events following pyridazinone application. Carotenoid synthesis inhibition may be the primary effect and a lack of β-carotene might relieve feed back inhibition of the pathway and allow precursors of its synthesis to accumulate [42]. The chlorophyll then cannot be protected and may be photo-oxidised. In untreated plants the thylakoid membrane contains the correct proportion of various lipids, pigments and other electron-carriers, and proteins. The imbalance brought about by the primary effect could regulate other synthetic pathways to produce secondary effects on lipids and both enzyme and structural proteins. In developing tissue this mechanism could also prevent chlorophyll formation.

We have already mentioned the fact that difunone (**15**) inhibits carotenoid synthesis in a similar manner to San 6706 and norflurazon [35]. Since these effects were brought about by growing the algae used in 1 μM difunone this is probably a better candidate for the primary effect than a direct influence on chlorophyll biosynthesis. Space will not permit more than a mention of other compounds probably acting primarily on carotenoid synthesis. These include dichlormate (**18**) and haloxydine (**19**) [1], aminotriazole (**20**) [47], fluridone (**21**) [45], and methoxyphenone (**22**) [48].

5. Concluding remarks

In sections 1 – 3 we considered interaction at known sites within electron transport and possible sites within the phosphorylation process. How can this information help us to make new herbicides? The general structural criteria for interference at a number of sites are known, but fairly vague. The obvious way forward is to make new compounds according to these criteria and to screen them for herbicide activity. Parallel *in vitro* tests can help through (i) guiding synthesis by indicating classes of compound which do not work *in vitro*, allowing synthetic effort to be directed towards compounds more likely to be successful, and (ii) highlighting compounds which work *in vitro* but not *in vivo;* this would lead to experiments or new synthesis directed towards delivering the demonstrated activity into the plant.

In contrast, the work in section 4 has concentrated on metabolic systems. Of necessity, most of the work has been done by looking for effects of a chemical in intact tissues, but it seems likely that, when the enzymology has been done, at least some of the compounds interfering with carotenoid biosynthesis will turn out to inhibit enzymes of the pathway. The biochemical knowledge might then be far enough advanced to attempt the design of inhibitors of other enzymes participating in carotenoid biosynthesis.

Acknowledgement

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