Structure-Activity-Relationship of Inhibitors of Photosynthetic Electron Flow

Herbicidal N-Alkylated-Ureas and Ringclosed N-Acylamides as Inhibitors of Photosystem II

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The inhibitory action of some herbicides on photosynthetic electron flow at photosystem II in isolated chloroplasts was investigated. Emphasis in the study is on compounds, whose chemical structure seemed to be in disaccordance with the basic structural element, proposed to be required for a photosystem II inhibitor. The effective inhibition of photosynthetic oxygen evolution by N-alkylated urea-, pyrrolidone- and by substituted pyridazine-derivatives without a free NH-group is reported. A revised basic chemical structural element responsible for inhibition is deduced in order to include lactames (ringclosed N-acylamides) with and without hetero atoms. From this new groups of potential inhibitors, like triazolone-, thiadiazolone- and oxadiazolone-derivatives may be conceived.

Photosynthetic electron transport of chloroplasts may be inhibited at various sites along the chain of carriers involved. Inhibitors at the electron acceptor site of photosystem II were the first to be discovered. Because many commercial herbicides are inhibiting photosynthetic electron flow at this site, their mode of action in isolated chloroplasts has been extensively studied (for review see l. c. [1–3]). The advantage of studying such herbicides with isolated chloroplast systems is that permeability barriers and transport problems through the different cell membranes and compartments to the point of action at or in the thylakoid membrane are not limiting and obscuring the actual inhibitory potency. Therefore with chloroplasts detailed information on the relationship of the extent of inhibition to the chemical structure of the herbicide may be obtained. The inhibitory activity obtained in a cell free system, expressed as pI_{50} = the negative logarithm of the concentration yielding 50% inhibition, can be related to extrathermodynamic parameters in way of a Hammet equation (Hansch approach [1]). From the comparison of the structure of inhibitors of photosystem II like ureas, anilides, triazines, uraciles, benzimidazoles, triazinones and biscarbamates it was concluded that a $-\overset{\overset{\|}{O}}{C}-NH$ (where C may be bound to O or N but not to S) is the basic chemical structural element in the herbicide responsible for its attachment to the inhibited site in the chloroplast membrane [1–3]. A lipophilic substituent at the nitrogen, usually an aromatic ring, is necessary for the approach to the inhibitor site. The third substituent on the carbon atom should be rather small (like $N(CH_3)_2$ in DCMU = 3-(3,4-dichlorophenyl)-1,1-dimethylurea) to prevent shielding of the essential element.

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The importance of the NH-group was particularly stressed, because N-alkylation led to inactive compounds as reported for ureas, benzimidazoles, triazines and biscarbamates (see review 2). This led to the speculation that a hydrogen bonding of the herbicidal structural element to a peptide bond in an enzyme at the inhibited site occurs [1]. However, examples of herbicides, or potential herbicides, have been described which are active to a small extent also with N-alkylated groups [2]. We wish to report on some striking examples of very active inhibitors of photosynthetic oxygen evolution which seem to be exceptions to the structural element formulated above. From this a revised basic element is proposed which allows to include further compounds in this group of photosynthesis inhibitors.

Results and Discussion

In Table I the effect of N-alkylation on the inhibitory action of certain substituted ureas on photosynthetic oxygen evolution in a Hill reaction by iso-

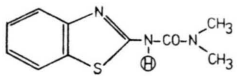
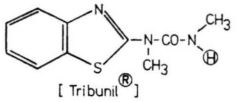
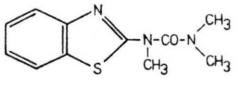
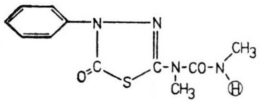
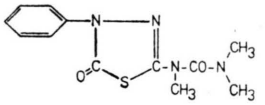
	50% inhibition obtained at y/ml	pI_{50}
benzthiazol-ureas		
	0.3	5.84
 [Tribunil®]	0.08	6.44
	0.27	5.84
thiadiazolone-ureas		
	0.023	7.06
	0.015	7.27

Table I. Influence of N-alkylation on the inhibitory effect of substituted ureas on photosynthetic oxygen evolution in isolated chloroplasts. Spinach chloroplasts were prepared according to Nelson *et al.* [6] and assayed at 15 °C under N_2 and illumination for 10 min with 30000 lx (Comptalux lamps). The medium contained in μmol in a volume of 3 ml: Tris buffer pH 8.0 80; $MgCl_2$ 10; ADP 10; inorganic phosphate 10; K-ferricyanide 20 and broken chloroplasts with 0.2 mg chlorophyll. Oxygen evolution was followed manometrically. Photosynthesis rates were in the order of 200 μmol oxygen/h and mg chlorophyll.

lated chloroplasts is summarized. The thiadiazolone- as well as the benzthiazol-ureas are as potent inhibitors of the reaction at a concentration of about 10^{-7} M as are the many other herbicidal ureas described in the past. As Table I indicates complete alkylation of the nitrogens in the urea moiety yields no decrease in inhibitory activity but actually to a small increase.

Another striking example of a potent inhibitor of photosynthetic electron flow with no NH-group is found in substituted pyrrolidones, which may be considered as ring closed N-alkylated acylanilides. Acylanilides are long known to be photosynthetic inhibitors (see reviews 1 – 3). The first compound in Table III indicates that a phenyl-pyrrolidone is a very effective inhibitor with a pI_{50} value above 6. Pyridazinone herbicides are also inhibitors of photosystem II as has been described already [4]. Moreland also observed that even the N-dimethyl derivatives of pyridazinones are still active inhibitors [4]. As Table II in addition indicates a pyridazinone derivative with no nitrogen at all in the side chain, but a methoxy group instead, is as active as inhibitor of photosynthetic oxygen evolution.

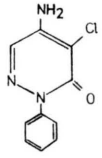
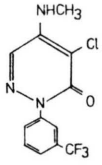
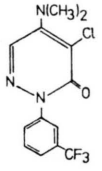
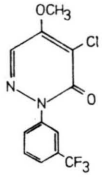
		50% inhibition obtained at y/ml	pI_{50}
Pyramin® [5-amino-4-chloro-2-phenyl-3-pyridazinone]		1	5.35
SAN H 9789		7	4.64
SAN H 6706		0.8	5.41
BASF 44521		1.4	5.23

Table II. Inhibitory effect of substituted pyridazinone derivatives on photosynthetic oxygen evolution in isolated chloroplasts.

This exceptions to the earlier rule that a free NH group is required for effective photosynthesis inhibition could be due to the possibility that either these inhibitors inhibit at a different site in the photosynthetic electron transport chain or that the structural element has been insufficiently described. A dealkylation mechanism might be envisaged in a whole plant, but is not likely to occur in the few minutes of incubation with the isolated thylakoids of the chloroplasts in the testsystem used here.

There is no indication of a change of the point of

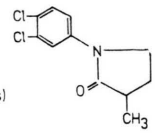
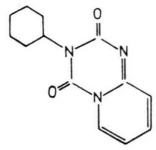
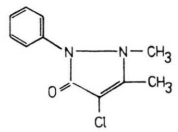
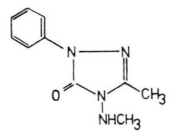
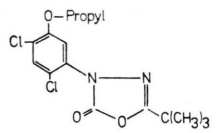
		50% inhibition obtained at y/ml	pI_{50}
Pyrrolidone (BV 207, Röhm & Haas)		0.2	6.09
Triazindione (Agripat)		3.3	4.77
Pyrazolone		8	4.25
Triazolone (Bay 143873)		4	4.7
Oxadiazolinone (17623 R.P.)		10	4.54

Table III. Inhibitory effect of some N-heterocycles on photosynthetic oxygen evolution.

inhibition in the electron transport chain by these compounds as judged by the effect of the inhibitors on part sequences of the chain (not documented here), *i. e.* artificial donor systems for photosystem II replacing the water splitting system are also inhibited. Therefore the second possibility has to be considered. That is if N-alkylation of the NH-group of compounds inhibiting the acceptor site of photosystem II does not necessarily lead to an inactive inhibitor then the concept of the active structure in this group has to be slightly revised. Hansch suggested an interaction of the bulky methyl group and the carbonyl oxygen of the protein at the active site in the chloroplasts [1]. Not only N-methylated compounds but also N-alkylated compounds with a large hydrocarbon side chain may be active inhibitors. Since even compounds with a heterocyclic ring as in the pyrrolidones are inhibitory, the explanation of Büchel [3] seems more appropriate, *i. e.* that it is the free electron pair at the nitrogen which is responsible for binding. This has the consequence that there is no hydrogen bonding of the inhibitor to the active (inhibited) site in the chloroplasts, as has been suggested [1, 2] but rather a hydrophobic interchange or a charge transfer interaction.

In the case of the pyridazinones it is apparent that the NH_2 group at the ring structure is not required for inhibitory action at all, because the O-substituted compound is as active (Table II).

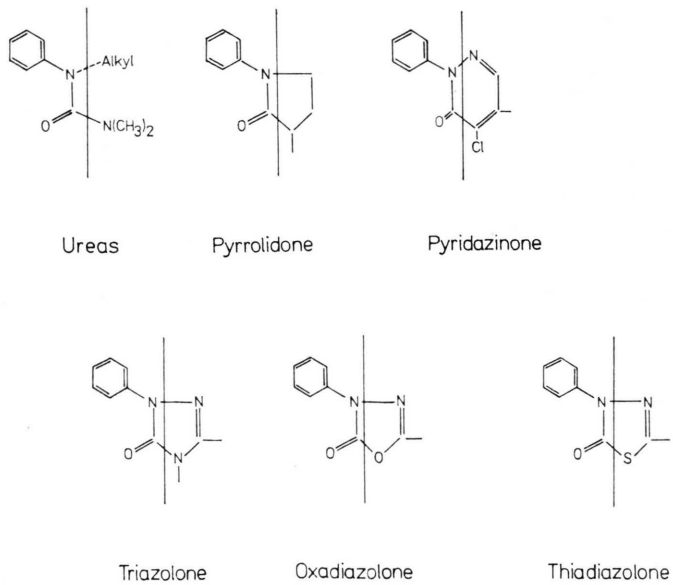


Fig. 1. Basic chemical structural element responsible for inhibition of photosystem II.

Therefore either not even a nitrogen is required in the active structure of herbicides of this group or, more likely, the active structure in the pyridazinone herbicides has to be sought in the ring structure itself. Rather than the structural element Hansch has proposed [1] for the pyridazinones the structure in Fig. 1 seems to be the active element.

From the formulation of this structural element in the pyridazinone herbicides other heterocyclic compounds with the same element can now be deduced to be inhibitors of photosystem II as well (Fig. 1). Though a phenyl-ring is indicated in this figure, another suitable substituent can be visualized.

This is indeed the case with the triazinone derivative Agripat which does not contain the decisive acylated amino sidechain of the triazine herbicides of the simazine type, but is nevertheless an active inhibitor (Table III). Also derivatives of triazolones are inhibitors of photosynthetic oxygen evolution (Table III) as are pyrazolones.

Oxadiazolones have been described as herbicides [5]. However, the compounds available to us are rather weak inhibitors of photosynthetic electron flow and only RP 17623 has some activity (Table III). The reason for their inactivity might be due, though, to the o-chlorosubstitution of the phenyl ring. As has been observed with several herbicides an ortho substitution close to the active element strongly decreases the inhibitory activity (see review 2). It might be expected therefore that 3,4-dichloro-phenyl-oxadiazolones will be active inhibitors of photosynthesis. The chemical structural element responsible for the inhibitory potency is indicated in Fig. 1. It becomes apparent now that the thiadiazolone-ureas (Table I) contain this element twice – in the urea configuration as well as in the heterocycle. This might explain the high inhibitory potency of this group of inhibitors. Of course, all the compounds, which were included in the earlier structure activity relationships *i. e.* the "simple" urea, triazine, anilide, uracil, benzimidazole and biscarbamate compounds still fit in the revised scheme of Fig. 1.

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